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Meeting report: 36th International Conference on Antiviral Research in Lyon, France – March 13–17, 2023

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Declaration of competing interest

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

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Abstract

The 36th International Conference on Antiviral Research (ICAR), sponsored by the International Society for Antiviral Research (ISAR), was held March 13–17, 2023, in Lyon, France, and concurrently through an interactive remote meeting platform. Here we provide a report summarizing the presentations at the 36th ICAR, including the ISAR speaker awards. We also detail special events, sessions, and additional awards conferred at the meeting. ICAR returned to in-person meetings in 2022, convening in Seattle, WA, USA. The 36th ICAR is the first in-person meeting of the society in Europe since the beginning of the COVID-19 pandemic, which restricted most events to virtual attendance to help mitigate the spread and subsequent public health impact of SARS-CoV-2. An exceptionally high number of registrants and record attendance at this year's ICAR, along with a vast array of demonstrable expertise in a variety of antiviral research-related fields, reflected a strong and growing antiviral research community committed to improving health outcomes from viral diseases, including SARS-CoV-2, and to future pandemic preparedness. This report highlights the breadth of expertise, quality of research, and notable advancements that were contributed by members of ISAR and other participants at the meeting. ICAR aims to continue to provide a platform for sharing information, fostering collaborations, and supporting trainees in the field of antiviral research. The 37th ICAR will be held in Gold Coast, Australia, May 20–24, 2024.

1. Introduction

The International Conference on Antiviral Research (ICAR) is the annual meeting of the International Society for Antiviral Research (ISAR). The 36th annual ICAR was held March 13–17, 2023, using a hybrid model with in-person sessions in Lyon, France, and on-demand virtual programming options. This was the second in-person ICAR since the SARS-CoV-2 pandemic began in late 2019. ISAR aims to provide an annual ICAR report as an overview of the conference based on speaker and event summaries contributed by a panel of society members (Andrei et al., 2017; Brancale et al., 2022; Bray et al., 2018; Spengler et al., 2023; Tramontano et al., 2019; Vere Hodge, 2003a, 2003b, 2011, 2013, 2014, 2015, 2017). The following report details speaker sessions, including the 2023 awardee lectures, and the results of the Chu Family Foundation awards as well as the poster and PechaKucha competitions.

ICAR 2023 began with the Women in Science panel discussion, followed by the opening session featuring the plenary speakers and the Gertrude Elion Memorial Award Lecture, the first of the ICAR award lectures. The rest of the award lectures were on subsequent days at the beginning of the speaker sessions. The speaker sessions featured invited and selected talks from international participants under the following subject areas: SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals; Chronic, Persistent, or Latent Viruses; Influenza, RSV, and Other Respiratory Viruses; and Acute GI Viruses.

Two poster sessions were held in person, the first during the evening of March 14th (the first full day of ICAR sessions) and the second over lunch the following day, providing additional advances in antiviral research. Special sessions and events included the PechaKucha competition, a tribute to Dr. Mike Bray (formerly editor-in-chief of *Antiviral Research* for 10 years), late-breaking oral presentations, the ISAR Annual Business Meeting, a career development interactive roundtable, and an evening closing award presentation event. Overall, ICAR again highlighted both the virological and chemical aspects of antiviral drug discovery, along with novel approaches to screening systems and disease modeling to facilitate these efforts. ICAR demonstrated antiviral research efforts from public and private partners in academia, industry, health care, and other national, international, governmental, and not-for-profit organizations in preventing and mitigating viral threats.

2. ISAR awards

2.1. 2023 Gertrude Elion Memorial Award. Discovery science for the cure of HBV infection: From the Understanding of Viral Persistence to Antiviral Targeting. Fabien Zoulim, Ph.D., INSERM, Lyon University, Hospices Civils de Lyon, Lyon, France

Fabien Zoulim (Fig. 1) presented his talk as the 2023 Gertrude Elion Memorial Lecture Awardee. This Award, supported by the Burroughs-Wellcome Fund, is given to an outstanding senior scientist who has made considerable contributions to the field of antiviral research and has a love of science, a reputation for scientific integrity, and approachability, especially to young scientists. Fabien began by explaining the disease burden associated with chronic hepatitis infections. Of the 296 million individuals living with chronic hepatitis infection, approximately 2 million people are treated and 820,000 people die due to cirrhosis and hepatocellular carcinoma (HCC) annually. Current treatments can suppress the virus, leading to decreased inflammation and fibrosis, which, in turn, slows disease progression. The reversal of fibrosis also decreases cirrhosis, reducing incidence of HCC.

Hepatitis B virus (HBV) is an enveloped, hepatotropic, non-cytolytic DNA virus belonging to the *Hepadnaviridae* family. Fabien summarized the HBV life cycle, which begins with binding to the sodium taurocholate co-transporting polypeptide (NTCP) receptor, allowing HBV to enter hepatocytes. The virus enters the cell and is trafficked to the nucleus; relaxed-circular DNA (rcDNA) is then released in the nucleus to form the covalently closed circular DNA (cccDNA). The cccDNA functions as a viral mini chromosome, serving as a template for all the viral transcripts. cccDNA is continuously replenished by intracellular recycling and is the key feature in viral persistence. In addition to cccDNA, double-stranded linear DNA may integrate into the host genome. Following transcription of cccDNA, pre-genomic RNA is encapsidated and reverse-transcribed into negative and positive strands. At this point, rcDNA contained in capsids can cycle back to the nucleus or become enclosed within envelope proteins to enter the secretory pathway and potentially infect new cells. Sub-viral particles, described as spheres and filaments, and soluble HBV e-antigen are also produced during the viral replication cycle. These key steps of the viral life cycle can serve as potential targets for developing antiviral drugs.

The HBV-affected liver faces a complex interaction of infected hepatocytes and immune cells, which determine the phases of infection. Within the liver, interactions of the virus

and host cells result in varying amounts of virus replication and inflammation, affecting the severity of fibrosis. The interactions between virus and cells also determine whether the disease evolves from one phase to another or reverts to a previous phase.

It has been demonstrated that individuals with chronic HBV infection have defective innate and adaptive immune responses. Prior research from Dr. Bertoletti's group in Singapore showed that T cell exhaustion was driven by the duration of high-level HBsAg expression. Fabien's group showed that intrahepatic innate response pathways are downregulated and influenced by HBsAg concentrations, which supports reduction of viral antigen expression as a mechanism for reviving innate immunity. The other determinant of viral persistence is cccDNA, which has a long half-life, is continuously replenished, and is not affected significantly by nucleoside (NUC) analogs or interferon treatment. Prior data from Fabien demonstrated that potent inhibition of viral replication by 2′,2′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine in the duck and woodchuck HBV models did not eliminate cccDNA. More recently, data from infected patients indicated that effective 30-year treatment with nucleoside analogs would be required to eliminate cccDNA.

Recent efforts have focused on evaluating endpoints for emerging therapies and achieving sustained off-therapy responses. The current goal is to obtain a functional cure defined by undetectable HBsAg and HBV DNA concentrations, normal ALT concentrations, persistently low cccDNA concentrations, and persistence of integrated viral sequences. An intermediate goal of a partial cure would have the following endpoints: low HBsAg concentrations, undetectable HBV DNA, normal ALT concentrations, persistence of cccDNA, and persistence of integrated viral sequences. Efforts toward this cure are focused on direct-acting antiviral drugs targeting viral entry, cccDNA formation, vRNA generation, capsid assembly or function, polymerase function, and HBsAg release. Drugs aiming to increase innate immune responses or restore adaptive immune responses are also being evaluated to aid in HBV clearance.

Fabien then summarized the clinical efforts to achieve a cure using combination therapy. He defined core protein allosteric modulators (CAMs) as drugs that enhance capsid nucleation. These compounds favor the formation of empty capsids or capsids with aberrant morphology. CAMs inhibit viral genome replication and prevent cccDNA formation in de novo infected hepatocytes. However, therapy with a CAM and NUC combination did not significantly reduce serum HBsAg in Phase II clinical trials. Several companies have also attempted to target the 3′ end of HBV transcripts (which is common to all transcripts) with siRNA. Combination treatment with a NUC and JNJ-2989 siRNA interference therapy decreased HBsAg followed by plateauing of HBsAg in clinical trials, a phenomenon which is not clearly understood. The HBV community has had to reassess a potential triple combination therapy to cure HBV, focusing on drugs that inhibit replication, reduce antigen levels, and stimulate the innate and adaptive immune system. Surprisingly, the triple combination treatment JNJ-73763989 (siRNA), JNJ-56136379 (CAM), and NUC in NUC-suppressed HBsAg-negative chronic HB patients performed worse than the dual combination of NUC and siRNA, and no patients achieved HBsAg sero-clearance. Other clinical trials have shown slightly different results. For example, the triple combination of CAM, siRNA, and NUC performed comparably to a dual combination of either CAM

and NUC or siRNA and NUC. A clinical trial evaluated siRNA VIR-2218 alone and in combination with PEGylated interferon alpha (IFNα) and found that 30% of patients achieved HBsAg sero-clearance. A quadruple combination ongoing study using siRNA, VIR-2218, and the neutralizing monoclonal Ab VIR-3434 in NUC-suppressed patients is showing a 3-$\log_{10}$ reduction in HBsAg; the study is still ongoing. Another study treated patients with chronic hepatitis B controlled by NUCs with bepirovirsen, an antisense oligonucleotide, and found significant reduction of HBsAg and HBV DNA that was maintained for 24 weeks after treatment. This strategy is now entering Phase III trials, the first time a new drug formulation has entered HBV Phase III clinical trials in the past 15 years. Several novel therapeutic vaccines for HBV to improve adaptive immune responses on the backbone of NUC therapy, with or without administration of checkpoint inhibitors, have also entered Phase I and II clinical trials.

Fabien then discussed the strategies that can be used to evaluate and target HBV infection within the liver compartment. New methods are needed to investigate the immunological and virological responses in the liver. Immunological biomarkers might highlight HBV-specific T and B cells, innate immune cells, or cytokines associated with infection. Non-invasive biomarkers are needed to assist clinical development of novel strategies. A greater understanding of cccDNA biology is also needed to allow direct targeting of this critical step in virus replication and persistence. Fabien's group evaluated the role of the HIRA histone chaperone in supporting cccDNA establishment and transcriptional infectivity. This work is ongoing, so future development may yield additional targets focused on achieving a cure for HBV. Understanding cccDNA decay and activity, as well as epigenetic changes associated with infection, may shed light on ways to target this step of infection. Despite the importance of cccDNA as an endpoint for evaluating the efficacy of HBV therapies, cccDNA is quantified using research assays, not commercially available assays. Fabien's group has participated in a collaborative consortium to standardize cccDNA quantification assays to make results comparable between studies. In addition to cccDNA, assays for non-invasive biomarkers, such as HBV DNA, circulating HBV RNA, HBV core-related antigen (HBcrAg), and HBsAg are needed for more comprehensive evaluation of HBV therapies. Even though HBV is a DNA virus, circulating HBV RNA is also found in infected patients and correlates with intrahepatic cccDNA in chronically infected HB patients. This indicates that circulating HBV RNA may be suitable for predicting the efficacy of HBV therapies.

Fabien identified three key concepts for curing HBV infection. He first questioned whether infected hepatocytes can be cured through viral targets or inducing an antiviral state. The second proposed concept to cure HBV is specifically killing infected cells by creating HBV-specific T cells or inducing specific cell death. The third proposed key concept was determining whether uninfected cells can be protected through virus neutralization or by inducing an antiviral state.

In conclusion, Fabien provided valuable insight on the current state of knowledge of the viral persistence mechanisms in patients with chronic hepatitis B and discussed drug discovery efforts that led to ongoing clinical evaluation of novel antiviral and immune strategies to cure HBV infection.

2.2. 2023 Antonín Holý Memorial Award. Tracking the Journey Towards the Discovery of Raltegravir and Grazoprevir: Two Intriguing Tales on Antiviral Drug Discovery. Vincenzo Summa, Ph.D., Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy

Thursday morning sessions started with one of the highlights of the meeting, the Antonín Holý Memorial Award talk. This Award, kindly sponsored by Gilead Sciences, is conferred to an outstanding senior chemist of international stature. The Award was instituted in 2014 and the previous honorees are Piet Herdewijn, Dennis Liotta, Bob Vince, David Chu, Chris Meier, Richard Mackman, Kathie Seley-Radtke, Eddy Arnold, and Mark von Itzstein. This year's awardee, **Vincenzo Summa** (Fig. 2), is without doubt an outstanding chemist of international statue, having developed two approved antiviral drugs: the HIV integrase inhibitor raltegravir (Isentress™) and the hepatitis C virus (HCV) protease inhibitor grazoprevir, commercialized in combination with elbasvir (Zepatier™). Vincenzo started his career at the IRBM Merck Research Laboratories in Italy in 1996 as a chemist, rising through the ranks to eventually become a director of the Medicinal Chemistry department. In 2009, he became a founding member of the IRBM Science Park, the spinoff of the Merck Research Lab in Italy, where he was appointed Vice President of Drug Discovery. He continues to seek new antivirals, especially agents to treat hepatitis B and Zika virus, and neglected tropical diseases. He became a full professor of medicinal chemistry at the University of Naples Federico II in the Department of Pharmacy in 2019.

Vincenzo thanked the committee for the Award and the opportunity to talk about the discovery of raltegravir and grazoprevir. He also underscored that the results presented were the outcome of a great collaborative and inspiring work between Merck teams in US and Italy that, in the spirit of finding the best possible molecules to give patients two very important antiviral drugs, always shared their ideas, failures, frustrations, and, eventually, successes. He then moved into his main topic by introducing the HCV and HIV antiviral landscape of 1996, when he started his career. At that time, there were no cell-based HCV assays and no structures of potential HCV targets had been solved. The only therapeutic available at the time Merck- IRBM decided to enter the field was PEG-IFNα plus ribavirin, which was only partially effective against one HCV genotype (gt1) and had many limitations. The anti-HIV field at the time was more advanced, with several nucleotide and non-nucleotide reverse transcriptase and protease inhibitors already approved; it was the dawn of the new era of highly active antiretrovirus therapy (HAART). Consequently, Merck was interested in new targets for developing drugs to use in combination therapies, and integration was considered a good potential target. It was amazing to realize how much progress has been made in the ensuing 16 years! Vincenzo briefly summarized the integration of HIV, emphasizing the mechanism of integrase. Two carboxylic groups coordinate two Mg^{2+} ions at the active site, which coordinate the creation of a nucleophilic attack on the phosphate of the backbone of the cellular DNA integration target to catalyze strand transfer. Several unrelated enzymes act via the same mechanism, like the HCV RNA-dependent RNA polymerase (RdRP), in which the two Mg^{2+} coordinate the attack on the alpha phosphate of the nucleotide to be incorporated, releasing pyrophosphate and adding the nucleotide to the growing RNA chain.

The first-generation integrase inhibitor identified had marked similarities with an HCV RdRP inhibitor, with a diketo acid group chelating Mg^{2+} at the active sites while the different hydrophobic moieties of each molecule conferred differential specificities for each target. These compounds were active against their target enzymes but had poor pharmacological properties. Based on these primary hits, Vincenzo's group designed related compounds using an alternative scaffold with similar Mg-chelating moieties, based on a dihyroxypyrimidine carboxylic acid template, but with greatly improved pharmacological profiles. A benzylamide derivative in this series surprisingly lost activity against HCV RdRP but gained activity against HIV integrase, which the researchers noted. Vincenzo's group used two main assays, integrase inhibition and spread inhibition, to test the inhibitors. As a target profile, they selected 100 nM in high serum conditions (50%), which more realistically replicates in vivo conditions. The early compounds lost activity in high serum but typically had much better pharmacokinetics. In contrast, the potency of other compounds changed markedly in enzymatic versus cellular antiviral assays but remained constant in high and low serum conditions. The researchers decided to start with the compounds with the best pharmacokinetic properties because increasing potency is easier than improving pharmacokinetics. The best compound had a good overall profile, but unfortunately showed CNS toxicity in mice and was thus discontinued; this was disheartening because it was the only active integrase inhibitor at the time. The group then derivatized compounds with worse pharmacokinetics, producing a N-methylpyrimidone carboxyamide derivative. The chemistry was more difficult, but they produced enough material to complete the development. Pharmacokinetic profiles in three different species predicted that this compound would be administered once per day, but unfortunately, it tested positive in the Ames test at very high concentrations, 3 g per plate - the compound was extremely soluble! Despite testing negative in all other toxicity tests, this compound, too, was discontinued.

A new series of compounds was developed by Merck US, a hybrid of diketoheterocycles and pyrimidines based on a naphthyridine scaffold. The series generated a lead compound that was tested in the rhesus macaque model of simian-human immunodeficiency virus (SHIV), showing proof of principle for using integrase inhibitors in vivo. It then cleared the 14-week preclinical safety study and moved into clinical trials. In a proof-of-principle test in one HIV patient, given as monotherapy for a limited period (which was allowed because the patient was infected with virus resistant to all then approved antivirals), it resulted in almost 100-fold viral reduction. However, at about the same time, the compound showed liver toxicity in dogs after prolonged exposure. Though this damage was not observed in rats, rhesus macaques, or humans, the program was again discontinued.

Vincenzo's group did not give up, though, and instead moved on to the next set of compounds, acylating the nitrogen in the N-methylpyrimidone carboxyamide series. They found an oxalamide and a constrained analog of it to be the best candidates. The constrained derivative had the best activity against most of the integrase inhibitor-resistant HIV mutants that they had selected during the development of all previous compounds. This compound was active, potent, and specific, with excellent pharmacokinetics; it cleared ancillary pharmacology and genotoxicity assays. Moreover, it was not metabolized by cytochrome p450, which is the main metabolism pathway for most other anti-HIV drugs, suggesting lower probability of drug interactions. The regimen was predicted to be twice daily. The

oxadiazol MK-0518 moved quickly into clinical trials and was highly active with favorable pharmacokinetics. It was active in treatment-naïve patients, as potent as the best alternative at the time, and was used as salvage therapy; it decreased HIV viremia to below 400 copies/mL in ~80% of patients when added to best therapies which alone could only achieve this decrease in ~20% of patients. This success resulted in its prompt approval for salvage therapy, and, later, for any HIV therapy; it became known as raltegravir (Isentress™). Vincenzo and his team were named Heroes of Chemistry in 2013 for this development. As they say, the rest is history.

Vincenzo then went back to HCV, focusing on NS3 protease inhibitors. No hits had been identified in high-throughput screens at the time. In 1996, Schering-Plough published the NS3 protease structure, showing a flat catalytic site, which was consistent with the lack of success in screens. As Vincenzo described it, it was like “climbing a flat rock.” Nonetheless, HCV protease is inhibited by its products, opening a path to antiviral development.
The minimal substrate was decapeptides, and the first identified hits were the processed product hexapeptides. While potency of these compounds could be improved and their size decreased, they were still nowhere near a pharmacological range. Based on this challenge, the group switched to covalent reversible inhibitors using a serine trap. The approach worked well, but the compounds were discontinued because of concerns about potential toxicity of covalent serine protease inhibitors. Vertex and Schering-Plough continued pursuing this approach and eventually produced telaprevir and boceprevir (as an aside, Vincenzo highlighted that the SARS-CoV-2 mPro inhibitor nirmatrelvir contains about 90% of the moieties in boceprevir). Vincenzo’s group instead went down a different path. The evaluation of the NMR structure of the HCV protease with its substrate suggested a strong possibility of a close contact between P1 and P3 and between P2 and P4, incentivizing the exploration of macrocycles. At the time, there were concerns regarding utilizing the P2/P4 proximity because the surface area of close proximity between these residues was on an exposed surface in the protease structure. Boehringer proceeded with P1/P3 cyclization and developed BILN-2061, which made it to the clinics before being discontinued due to cardiotoxicity. Nonetheless, BILN-2061 proved to be a potent reversible antiviral inhibitor, demonstrating the utility of non-covalent HCV protease inhibitors. The approach followed by Boehringer’s group required a large hydrophobic moiety in P2, which was surprising because that was supposed to be an exposed surface. However, co-crystallization of the full-length NS3 protease with the NS3 helicase showed that this “exposed” surface was actually buried in the interacting surface. That observation triggered the re-evaluation of cyclizing P2-P4. The P2-P4 macrocycle vaniprevir then progressed rapidly. The compound is very potent and concentrates in the liver but cannot be detected in plasma, which raised the challenge of developing a compound that cannot be clinically followed. Vincenzo’s group needed an animal model to validate, showing that vaniprevir decreased viremia by ~5 logs in chimpanzees. It was clinically active, again reducing viremia by 5 logs, but was not active against HCV genotype 3 (being specific to genotype 1) and lost potency with the two most relevant resistant clinical mutations. Working on the crystal structure, the group opened one of the heterocycles and expanded towards the more conserved regions of the protease, producing a compound active against the most common mutations and genotypes and with a much higher resistance barrier. This compound was detectable in plasma,

although liver concentrations were 100-fold higher than with vaniprevir, facilitating its clinical development. The new compound was potent in chimpanzees and had pangenotypic activity. It was named grazoprevir. Grazoprevir was then formulated in combination with Merck's own independently developed NS5 inhibitor elbasvir, which had been approved for treatment and cure of HCV genotypes 1, 3,4, and 6. Vincenzo and his team, together with the team developing elbasvir, were once again named Heroes of Chemistry in 2017. Vincenzo emphasized that this success was the result of a "gigantic" effort across the world, with contributors in the US, Italy, and Japan, and he thanked them all for their great effort.

Vincenzo's talk thus described a true tour de force in antiviral development, with many false starts and dead ends that were all eventually overcome by smart and hard work, curiosity, and unwavering support to reach the ultimate goal of developing drugs to help the millions affected by HIV or HCV.

2.3. 2023 William Prusoff Memorial Award. Preparing for Tomorrow's Pandemics Today Through the Development of Broad-Spectrum Antivirals. Timothy P. Sheahan, Ph.D., University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

The William Prusoff Memorial Award is given annually to an outstanding young scientist who has demonstrated dedication and excellence in the field of antiviral research (basic or clinical, synthetic or pharmacological) as well as future potential for contribution to the field and the society. The 2023 award was given to **Timothy Sheahan** (Fig. 3), an assistant professor from the University of North Carolina at Chapel Hill.

Coronaviruses (CoVs) have great emergence potential, often spilling over from one species to another to cause new diseases. In the past 20 years, such spillovers have occurred at least three times. SARS-CoV-2, the cause of COVID-19, is the most recently emerged human CoV, but given the emergence and pandemic potentials of CoVs, these viruses are likely to continue emerging in the future. Thus, potent, broadly active antiviral therapies are needed to maximize our pandemic preparedness. Timothy's team has developed several in vitro and in vivo models of CoV replication and disease to assess the efficacy of antiviral treatments. Prior to the COVID-19 pandemic, the team used these models to evaluate the therapeutic potential of remdesivir and molnupiravir for emerging CoV infections. These studies demonstrated the potential of these agents for treating CoV infections, which helped expedite their clinical testing in early 2020. They continue this strategy with SARS-CoV-2-specific tools to provide pre-clinical data for various antiviral therapies, such as the main protease (M^{pro}) inhibitor NZ-804. The lab's future work aims to develop combination antiviral therapies that reduce the potential for antiviral resistance and mitigate the pathogenic components of the host response.

Before the pandemic, no vaccines or therapies were approved for any human CoV infections; now several approved vaccines and antiviral therapeutics are available. Timothy's and his collaborators' heroic efforts really demonstrate the power of antiviral research, which has enhanced our preparedness for future emerging CoV, and provide a blueprint for rapid antiviral response to future emerging viral diseases.

2.4. 2023 Women in Science Award. Structural Studies Facilitate Antiviral Drug Development Targeting the SARS-CoV-2 Main Protease and Variants of Concern. Joanne Lemieux, Ph.D., University of Alberta, Edmonton, Alberta, Canada

The Women in Science Excellence Award is the second newest award of the Society, instated in 2017. Previous awardees were Drs. Priscilla Yang, Desiree LaBeaud, Grace Zhou, Anna Wald, Graciela Andre, and Christina Spiropoulou. This year's awardee was **Joanne Lemieux** (Fig. 4). Joanne started her talk by thanking the Awards committee and her nominator, Dr. David Evans (University of Alberta), and stated that she was planning to attend ICAR before learning of her award, but she was even happier to attend as the awardee. She next introduced the team that had participated in the presented work, emphasizing the participation of the chemists.

Joanne briefly discussed the main role of the Nsp3 and Nsp5 proteases of SARS-CoV-2. Nsp3, the papain-like protease (PL^{pro}), cleaves the polyprotein at only three sites, whereas the main protease Nsp5 (M^{pro} or $3L^{pro}$) cleaves it at all others. Based on this difference, the group focused their work on M^{pro}, even though Joanne had previously worked with membrane proteases. M^{pro} has a strong preference for glutamine in P1 and leucine in P2. Besides cleaving SARS-CoV-2 polyproteins, M^{pro} cleaves cellular proteins, among which Joanne focused on galectins, lectins that bind to galactose-containing glycans and are normally involved in sensing endosomal damage.

After this introduction, Joanne highlighted the many therapeutic uses of protease inhibitors in treating cardiovascular disease, diabetes, and viral infections, and the current focus on finding SARS-CoV-2 M^{pro} protease inhibitors that require no ritonavir boost. Her group's first approach was to evaluate the activity of GC376, an inhibitor of the FIV protease, which was active against SARS-CoV-2 M^{pro} in vitro and in cell culture with an EC_{50} just below 1 μM. The FRET-based assay that Joanne's group uses for in vitro evaluation is more sensitive than other assays, being capable of detecting the difference in protease activity between SARS and SARS-CoV-2 proteases that peptide-based assays do not; these differences result in different IC_{50} absolute values. The group solved the co-crystal structure, which Joanne discussed in detail, highlighting that the P3 position did not establish many interactions with the inhibitor. Rational drug design was used to increase these interactions to reach IC_{50} of 17 nM and EC_{50} (in cell culture) of ~0.5 μM, which was decreased by P-glycoprotein multidrug transport inhibitors to just below 200 nM. The group also focused on de novo design of protease inhibitors, using the knowledge of the already existing inhibitors of rhinovirus, norovirus, and SARS proteases and exploring both reversible and irreversible covalent inhibitors. Joanne first described an irreversible inhibitor with a modified warhead including a six-atom heterocycle instead of the usual five-atom one. Considering the potential for side effects, her group evaluated the selectivity of this inhibitor for M^{pro} over cathepsins; it showed ~100-fold higher selectivity for the former. The compound was also active against coronaviruses 229E and OC43 but was limited by cell efflux and microsomal clearance. To sidestep the potential toxicity of irreversible inhibitors, the group switched the warhead to a nitrile group. Using this approach, they produced compounds with EC_{50} in the submicromolar range. They solved the crystal structure of the best of these compounds, which, unsurprisingly, showed interactions similar to those of the irreversible inhibitor on

which it was based. The researchers continued improving the compounds, developing a new series with a better pharmacokinetic and pharmacodynamic profile in hamsters, comparable to or better than the profile of nirmatrelvir. Animal studies had just started at the time of the talk and results were not yet available.

Most M^{pro} variations occur in alpha, gamma, and delta SARS-Co-2 strains but are seldom present in beta or omicron strains. These variations were mapped to three hot spots: one close to the active site, another behind it, and the third in the dimerization surface, a domain that differs between SARS and SARS-CoV-2. Thirty-one mutant proteases were produced and purified. Most mutations affected M^{pro} kinetics, with substitution F185S resulting in a dead protease by disrupting dimerization. Joanne proposed that this mutation, which is predicted to result in a non-viable virus, was likely in a quasispecies in a patient infected with a virus with variants of functional protease. Different variations at position E47 increased or decreased protease activity, and some variations altered peptide substrate specificity without affecting the absolute leucine preference of P1. Eleven M^{pro} variants with different enzymatic activity were crystallized. The molecular bases for the different activity and substrate specificity could be identified for the hotspots around the catalytic site and behind it, whereas the mutations at the dimerization surface surprisingly did not have much of an effect.

Joanne's group proceeded to evaluate the degradation of galectin 8 by M^{pro}, stopping the digestion at different times with one of their protease inhibitors. Three cleavage sites were identified in galectin 8, one of which was not in the linker region. In contrast, galectin 9 was not cleaved. (Joanne mentioned that she was already working on galectins in a collaboration before the pandemic.) Interestingly, some of the M^{pro} variants were more or less active against galectin 8 as confirmed by kinetic analyses. Most of these variants were from gamma or delta SARS-CoV-2 strains, but not from omicron. To determine physiological effects of galectin M^{pro} cleavage of galectin 8, the researchers exposed PBMCs to full-length galectin or a mutant galectin 8 truncated at the cleavage site. The truncated galectin decreased cytokine secretion (specifically of TNF). In contrast, none of the variations had a significant effect on sensitivity to their inhibitor or nirmatrelvir. Joanne concluded that these data support the hypothesis of a protease envelope, which is consistent with a recent publication by the Schiffer group showing that binding of all peptides cleaved by M^{pro} in the SARS-CoV-2 polypeptide have a common footprint in the protease. Joanne finished by thanking a number of collaborators and funding and announcing that her group had just received funds for a pandemic Hub, which is the Canadian equivalent of the USA AViDD centers.

In summary, Joanne gave an excellent talk describing the development of novel SARS-CoV-2 protease inhibitors. Excitingly, some of the presented results are now published in *ACS Central Science*; the manuscript was accepted on the day that Joanne gave her talk.

2.5. 2023 Diversity Award. How a Love of RNA Biophysics Led to the Discovery of a Novel Antiviral Against Enteroviruses. Blanton S. Tolbert, Ph.D., Case Western Reserve University, Cleveland, Ohio, United States

Blanton Tolbert (Fig. 5) began his lecture by introducing the importance of RNA-protein interactions in mediating viral pathogenesis, and how these numerous intersections between

host proteins and viral RNA (vRNA) represent untapped potential targets for therapeutic intervention. He introduced his work with enterovirus-A71 (EV-A71), the etiological agent of hand, foot, and mouth disease for which there are currently no FDA-approved vaccines of antivirals. As a positive-sense RNA virus, EV-A71 translation is driven by a 5′-UTR IRES element that has been shown to interact with numerous cellular and viral proteins, including the hnRNP family of cellular proteins that are frequently usurped by RNA viruses like EV-A71 to modulate viral translation and genome synthesis. Contained within the IRES element is a stem loop II (SLII) domain that is highly conserved between enteroviruses, and Blanton described his work identifying the mechanisms by which the mutually antagonistic hnRNP A1 and AUF1 proteins compete for the SLII structure to differentially regulate enterovirus translation efficiency. He presented data showing that mutations in this SLII-IRES domain inhibited viral replication by altering SLII topology, which impacted translational efficacy. Next, his group screened a library of small molecule RNA binders and identified the compound DMA-135, which was shown to bind SLII to dose-dependently inhibit viral replication by attenuating viral translation. Serially passaging EV-A71 in the presence of low doses of DMA-135 selected for resistant viruses with suppressor mutations, mapped to the SLII bulge environment, that negated the antiviral effect of DMA-135. Comparative structure-function studies revealed that the cellular mechanism of action of DMA-135 is to tip the SLII-hnRNP regulatory axis towards significantly lower levels of IRES-dependent translation, and the virus can compensate by evolving mutations that restore homeostasis. Blanton concluded his lecture by discussing how these data show that the architecture of a protein-RNA complex can influence biological function, and how viral RNA structures can be targeted by small molecules to both better understand vRNA-host interactions and to serve as antivirals by inhibiting viral translation.

3. ISAR plenary speakers

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

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

Esteban Domingo discussed the concept of quasispecies in the context of antiviral resistance. His talk began by focusing on the complexity of viral populations, comparing the consensus sequences to the mutant spectra. When viruses infect an organism, they develop a mutant swarm, or mutant spectrum, with several mutations throughout the genome driven by high mutation rates. The consensus sequence is an average of all sequences present in the sample but may not even be represented in the actual population. For some viruses, the mutation rate can reach 1×10^{-4} mutations per nucleotide copied. Viral replication drives the population to become more heterogeneous. Recombination and genome segment reassortment also increase genetic diversity. Esteban’s laboratory evaluated SARS-CoV-2 and showed that virus populations contained both mutations and deletions within genomes; deletions within the genome are rarely seen in other viruses. Quasispecies dynamics are categorized as three separate implications ranging from theoretical to more applied. The first implication is that the “wild-type” genome is a set of genomes rather than one specific

sequence. The second implication is that viral adaptability is explained by mutant spectrum dynamics. The third implication is the influence on antiviral interventions, including the development of lethal mutagenesis. Quasispecies research has demonstrated that the viral spectrum contains many genomes and within that subpopulation are viruses that differ in critical phenotypic traits. The mutant spectra are then phenotypic reservoirs for the virus population. A complication to interpreting viral evolution is the frequent occurrence of bottlenecks, where scientists sample a portion of a viral population but cannot be sure whether the sample represents the mutant spectrum of the virus. This is affected by the sample of virus and its adaptation to new hosts. Esteban's laboratory serially passaged HCV 200 times in cell culture and demonstrated a 2.2-fold increase in fitness compared to the initial virus isolate. Analyses by self-organized maps of haplotypes identified by deep sequencing data showed that long-term replication of HCV in Huh-7.5 cells shifted haplotype peak positions in the absence of external selective pressures, documenting absence of population equilibrium.

Esteban proposed that quasispecies dynamics should be considered during vaccine and antiviral design. To limit adaptive changes, viruses should be confronted with multiple selective pressures simultaneously to present an unbearable fitness cost for the virus. To accomplish this goal, vaccines should be complex (multiepitopic) and antiviral compounds should be potent and administered in combination. Work with HIV and HCV demonstrates the success of combination therapies. Esteban's group has approached this concept by investigating lethal mutagenesis as an application of the error threshold concept of the quasispecies theory. Decreased replication fidelity leads to lethal defects, in which the created defective genomes interfere with viral replication. Further decreases in fidelity lead to overt lethality and eventually violation of the error threshold and loss of infectivity. Longer viral genomes lower the virus's ability to support genome mutations.

Synergistic combinational antiviral therapies reduce side-effects due to lower drug dosages and minimize the selection of escape mutants. Synergism may be reinforced by differences in mutation types as well as preference for mutations sites. Synergistic lethal mutagenesis of HCV has been documented with favipiravir and ribavirin in cell culture.

Esteban then discussed how low viral fitness and low viral load favor virus extinction. Since viruses increase in fitness when replicating in the same environment, his laboratory hypothesized that viruses should be more difficult to inhibit with antiviral therapy during ongoing infection. To demonstrate this concept, they used combinations of daclatasvir + sofosbuvir and favipiravir + ribavirin to treat low- and high-fitness HCV at different times after the onset of infection. For both combinations, delaying the addition of inhibitors reduced their antiviral efficacy, but the effect was far more pronounced for high-fitness HCV. These data support the idea that antiviral treatment should "hit early and hit hard" to best decrease viral fitness.

The next question Esteban addressed was whether SARS-CoV-2 is able to generate mutant virus swarms despite having a 29 kB genome. His group found an abundance of low-frequency mutations in SARS-CoV-2 mutant spectra compared to other RNA viruses. Complexity of the spectrum was higher in samples obtained from patients that developed

mild rather than moderate or severe disease. Another study showed that breakthrough viruses from patients infected with the alpha variant contained mutations from variants that emerged much later in the pandemic (e.g., delta plus, iota, and omicron variants). The group determined that SARS-CoV-2 replicates as complex mutant swarms and that minority substitutions can be found in consensus sequences of earlier or later virus isolates. They concluded that the challenges posed by developing antiviral therapies to SARS-CoV-2 were not significantly different from challenges posed by other RNA viruses.

Esteban's group evaluated the combination of remdesivir and ribavirin and showed a synergistic effect on SARS-CoV-2 in cell culture. Similar results were obtained using the seasonal human alphacoronavirus 229E.

Esteban summarized his talk with the following points. First, dynamic mutant spectra are the substrate for the adaptability of RNA viruses. Second, effective treatments should consist of confronting the virus with multiple fitness-reducing selective constraints. Third, synergistic lethal metagenesis offers a way to evaluate broad-spectrum antiviral interventions by determining the effect on mutation type and mutation site preferences. Fourth, SARS-CoV-2 is not an exception to the complexity challenge, and deep sequencing has revealed a huge minority genome reservoir in populations of this virus. Last, the synergism demonstrated by remdesivir and ribavirin, together with synergisms identified in other studies, supports the idea of exploring synergistic lethal mutagenesis as a strategy to treat severe COVID-19.

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

Laurent Fraisse explained that the Drugs for Neglected Diseases initiative (DNDi) was created in response to the frustration of clinicians and desperation of patients that were either provided with medicines that were ineffective, unsafe, unavailable, or unaffordable, or with nothing at all because, in some cases, drugs for their diseases had never even been developed. Our current model for medical research does not incentivize the development of drugs for the poorest communities despite the clear medical need. Since its founding in 2003, DNDi has operated as a global partnership, linking leading research institutions together as a non-profit with a focus on developing medicines for low- and middle-income countries. DNDi accomplishes this through the following three pillars of their mission: innovate to save lives, foster inclusive and sustainable solutions, and advocate for change. Over the past 20 years, they have developed 12 field-adapted and affordable treatments for six deadly diseases. These efforts have saved lives and had a significant positive impact in delivering access to urgently needed medical treatments to neglected patients.

Laurent focused first on HIV, which is not typically considered a neglected disease except when it comes to children. Currently, 1.7 million children under the age of 14 are living with HIV globally; 84% of them live in sub-Saharan Africa. As of 2021, only 52% of these children were on HIV treatment, and 98,000 children under the age of 15 died of AIDS-related causes in 2021. In 2013, DNDi focused on overcoming drug-drug interaction in young children who were being treated for both HIV and tuberculosis (TB). DNDi's super-

boosting study in South Africa evaluated treatment with lopinavir/ritonavir in children co-infected with TB/HIV and concurrently receiving rifampin-based therapy for TB. The results informed a 2016 World Health Organization recommendation on super-boosting ritonavir when treating TB with rifampin-based therapy in TB/HIV co-infected children receiving lopinavir/ritonavir-based anti-retroviral therapy. Beginning in 2014, DNDi also developed a taste-masked, fixed-dose anti-retroviral therapy formulation to replace the awful-tasting syrups that are difficult to administer to very young children. They created a strawberry-flavored capsule containing abacavir/lamivudine/lopinavir/ritonavir (4-in-1) that could be sprinkled on foods or suspended in liquids. In an earlier clinical study in Kenya, Uganda, and Tanzania, DNDi found that 2-in-1 lopinavir/ritonavir pellets with abacavir/lamivudine tablets were well tolerated and well accepted by children under 24 months of age with advanced/severe disease. In a second study, the 4-in-1 formulation itself was shown effective and was described by 97% of caregivers as very easy or easy to administer compared to previous formulations. Although necessary, drugs alone are not enough. Treatment access plans are also needed to ensure that effective drug formulations can be accessed by children in need.

Laurent then shifted his talk to HCV, one of the few infectious diseases that could potentially be eliminated globally with the tools that exist today. Despite the availability of direct-acting antivirals that can cure patients, access to these treatments is limited in low- and middle-income countries where 75% of the world's people infected with HCV reside. In 2016, DNDi partnered with Presidio Pharmaceuticals (US) and Pharco Pharmaceuticals (Egypt) to develop ravidasvir, an oral inhibitor of HCV replication. This compound has promising clinical efficacy and was combined with sofosbuvir; plans are to make it available and affordable in countries where treatment costs for HCV remain high. The STORM-C-1 clinical trial to evaluate the ravidasvir/sofosbuvir combination demonstrated efficacy in all tested genotypes and a high cure rate for cirrhotic patients with genotype 3, which is particularly difficult to cure. In addition, no significant drug-drug interactions were observed for patients with HIV co-infection and currently taking anti-retroviral therapies.

Recent efforts at DNDi have focused on creating a coalition to ensure that COVID-19 clinical research includes the participation and meets the needs of resource-limited settings. DNDi is working to review the best therapeutic candidates to take forward for clinical testing against COVID-19. In 2020, the ANTICOV clinical study in 13 African countries and Brazil evaluated the safety and efficacy of several therapies for mild to moderate cases of COVID-19. The waves of COVID-19 infection made evaluating these therapies difficult. Importantly, this clinical trial demonstrated the ability to effectively contribute to the control of future emerging pandemics through a flexible clinical research program (platform trial) with a focus on low- and middle-income countries. DNDi also aided in the establishment of an open science initiative, COVID Moonshot, to develop a clinically validated therapeutic against M^{pro} of SARS-CoV-2 with the goal of equitable global access. Collaborators used a structure-based design from x-ray structures and computational chemistry to develop an advanced lead compound with low micromolar efficacy against the SARS-CoV-2 M^{pro}. This compound demonstrated broad antiviral activity across the SARS-CoV-2 variants. A second initiative supported by NIH (AViDD center, ASAP: AI-driven Structure-enabled Antiviral

Platform) dedicated to the search for new antivirals against future pandemics has been initiated with the contribution of DNDi.

Laurent also highlighted dengue virus as an unmet medical need since it is the most prevalent mosquito-borne viral disease in the world but has no existing treatment. With 394 million people infected annually, there is an urgent need to treat dengue fever and prevent progression to severe disease. A dengue alliance was formed with institutions from endemic countries to develop a treatment and establish a clinical network to provide proof-of-concept trials. The planned work will also improve dengue fever epidemiology and modelling knowledge for Africa, where the gaps in epidemiological data are substantial.

In conclusion, DNDi has been involved in addressing viral infections within neglected patient populations. They are establishing innovative scientific partnerships to focus on neglected populations to enable identification and affordable treatments for dengue, COVID-19, and future pandemic diseases.

4. Session 1: SARS-CoV-2, arboviruses, other biothreat viruses, and broad-spectrum antivirals I

Chaired by Kathie Seley-Radtke, Luis M. Schang, Dahai Luo, and María Jesús Pérez Pérez.

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

Although effective vaccines are available, yellow fever remains a deadly threat to people in endemic regions worldwide. **Jinhong Chang** reported on an acetic acid benzodiazepine compound (BDAA) discovered in a cell-based high-throughput screen that specifically inhibits yellow fever virus (YFV) replication. A resistance mutation was mapped to the YFV non-structural protein 4B (NS4B). Through a well-designed structure-activity relationship study, the team identified lead candidates that showed improved druggable properties and provided 100% therapeutic protection against lethal YFV infection in a hamster model. The team then conducted extensive mechanistic studies to further understand how the drug candidates work to inhibit YFV. Treatment with BDAA induces significant ultrastructure alterations in the viral replication organelles (ROs) and the exposure of dsRNA in the cytoplasm of virus-infected cells. These alterations are associated with the enhancement of the YFV-induced inflammatory cytokine response. These results support a model in which BDAA interaction with NS4B impairs the integrity of YFV RO, directly abrogating viral genome replication and promoting viral replication intermediates releasing from RO to activate cytosolic RNA-sensing pathways. BDAA directly inhibits nascent YFV RNA synthesis in cell culture in a 5-ethynyl uridine incorporation experiment and in an endogenous polymerase reaction system using isolated ROs. Furthermore, BDAA treatment activates three major dsRNA recognizing pathways, RLR, PKR, and OAS-RNase L, in YFV-infected cells. The work shows that BDAA primarily targets the YFV RO and executes unprecedented multi-mode antiviral action that may collectively lead to rapid-acting and potent inhibition of viral replication in vivo. This study advanced the preclinical

development of this family of antiviral compounds and prepared them for future clinical studies, offering hope for a new treatment option for yellow fever.

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

Marnix Van Loock was honored with the 2019 William Prusoff Young Investigator Award from the International Society for Antiviral Research for his team's groundbreaking work on developing an antiviral small molecule to combat dengue, a significant global medical issue. During his presentation, Marnix discussed the journey of discovering and developing the dengue antiviral molecule, which is currently in clinical development, and demonstrated the importance of public-private partnerships in research and development. He outlined the different stages of drug discovery and development, highlighting the contribution of each partner to the process. This successful model set a good example for developing antivirals against other neglected and emerging infectious diseases. Through years of collaboration across various sectors, two antiviral compounds, JNJ-A07 and JNJ-1802, were discovered, with the latter advancing to clinical development. JNJ-1802 specifically targets the replication of dengue virus by inhibiting intermediate steps during the assembly of the virus replication complex. Clinical trials are currently ongoing to assess the safety and effectiveness of JNJ-1802 in humans.

4.3. The Mechanism of RNA Capping by SARS-CoV-2. Vincent Tagliabracci, Ph.D., HHMI/UT Southwestern, Dallas, Texas, United States

During his talk, **Vincent Tagliabracci** discussed the structural and mechanistic basis of the RNA 5′-end capping process in coronaviruses. The SARS-CoV-2 genome contains a 5′ cap that is crucial for viral protein translation, immune evasion, and protection from exonucleases. To gain insight into this capping, Vincent's team reconstituted the cap structure (7MeGpppA2′-O-Me) using viral non-structural proteins (Nsps). They found that the kinase-like nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain of Nsp12 transfers the RNA to Nsp9, forming a covalent RNA-protein intermediate called RNAylation. The NiRAN domain then transfers the RNA to GDP, creating the GpppA-RNA core cap structure. The Nsp14 and Nsp16 methyltransferases add methyl groups to form functional caps. Structural analysis of the replication-transcription complex bound to nsp9 identified key interactions involved in the capping reaction. The team's reverse genetics system showed that the NiRAN domain's kinase-like active site residues and Nsp9 N-terminus are required for successful SARS-CoV-2 replication. This study revealed an unconventional capping mechanism, exposing a new target for developing antivirals against COVID-19. It also highlights the versatility of the protein kinase fold in viruses, prokaryotes, and eukaryotes, linking back to their previous work on pseudokinases.

5. Session 2: chronic, persistent, or latent viruses I

Chaired by Jinhong Chang, David Durantel, Kara Carter, and Fabien Zoulim.

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

In the first session of the Chronic, Persistent, or Latent virus section, four state-of-the-art presentations were given. The first, given by **Thomas N. Kledal**, concerned cytomegalovirus (CMV) infections in immunocompromised patients, which can be notably problematic after transplantation, leading to significant morbidity and mortality and increased readmission rates, and increased overall cost of transplantation. Current standards of care (SoC) have some value in protecting against lytic infection, but no approved antiviral targeting the latent phase of CMV infection exists. SYN002 is a fusion toxin protein able to specifically target the CMV-encoded chemokine receptor US28 and to induce death of actively or latently infected cells in vitro. While this biopharmaceutical is progressing its way to further clinical evaluation, Thomas showed that, in ex vivo human lung perfusion experiments, a 6-h administration of SYN002 through the pulmonary artery markedly attenuated CMV reactivation with no acute toxic events based on physiology and quantification of cytokines. The ability of SYN002 to target latency, combined with its fast mechanism of action, hold therapeutic potential, including for targeting CMV in organs ex vivo prior to transplantation.

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

Hepatitis D virus (HDV), the causative agent of the most aggressive viral hepatitis, relies on hepatitis B virus (HBV) envelope proteins to release infectious particles. Due to its HBV dependency, compact genomic organization, and lack of HDV-encoded enzymatic activities (except HDV ribozyme, which is hardly druggable), HDV offers very few direct therapeutic targets. During her broad and general lecture, **Maura Dandri**, who has pioneered HDV studies in liver-humanized mice (HuHep), specifically updated the audience on the clinical use of anti-HDV drugs. PEGylated IFNα, an “old” innate immune stimulator and drug, has been until recently used as backbone SoC for HDV infection, although used off-label. Besides the fact that IFNα is poorly tolerated, not all patients are sensitive to IFN therapies and the reasons for this (host genetics, presence of anti-IFNα, etc.) are currently being investigated. Maura’s team found that sensitivity to IFNα varied according to HDV genotypes/subtypes, with HDV-1a being far less sensitive than HDV-1p or HDV-3 in HuHep mice. Moreover, in vitro and in HuHep mice, HDV can induce (through MDA5/LGP2 sensing) an endogenous IFN response that could contribute to liver inflammation and interfere with therapeutic efficacy of IFNα. A better understanding of HDV/host cells interactions should be instrumental in identifying patients more prone to respond to IFN therapy or to find and develop alternative immune-based therapeutic options. Bulevirtide (an HDV entry inhibitor targeting NTCP receptor) has obtained conditional approval by EMA in 2020. Intrahepatic analyses performed in paired liver biopsies (at baseline and 48 weeks) from clinical trials MYR203 and MYR301 showed a strong dose-dependent decline of intrahepatic HDV markers. Moreover, HDV decline was associated with decreased inflammatory gene expression, indicating reduction of liver inflammation.

In combination with pegIFNα, BLV appears to augment antiviral efficacy. However, the underlying mechanism of this synergy needs further investigation in preclinical models.

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

Eric Lansdon reminded us that emtricitabine (FTC) and lamivudine (3 TC) are widely used nucleoside reverse transcriptase inhibitors (NRTIs) in antiretroviral therapy for HIV. Both drugs contain an oxathiolane ring with unnatural (−)-stereochemistry (*L*-nucleoside) as a mimic of ribose found in natural deoxynucleosides. Pre-steady-state kinetic measurements with HIV-1 reverse transcriptase (RT) illustrate that (−)-FTC triphosphate (TP) and (−)-3 TC-TP have a higher binding affinity than dCTP, but their rate of incorporation is slower. Taken together, the overall selectivity factor of these drugs is comparable to that of dCTP. Crystal structures of the HIV-1 RT/dsDNA complex showed how RT recognizes these drugs as substrates and explained their slower rate of incorporation. The resistance mutation M184V was also introduced into the RT, and pre-steady-state kinetics showed that the mutation lowers the binding affinity for *L*-nucleosides but does not affect their rate of incorporation. Crystal structures highlighted that the M184V side-chain has no room to move in the pocket in response to different substrates. Furthermore, the crystal structure of RT with M184V and (−)-FTC-TP showed a steric clash with the oxathiolane ring in the binding pocket, which lowers the Kd compared to that of dCTP and discriminates against *L*-nucleoside binding. This knowledge is instrumental for identifying *L*-nucleosides less prone to mutation selection both in the HIV and HBV fields.

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

Pro-drugging has become an important strategy in drug design and development, with around 10% of all marketed drugs considered as prodrugs (PDs). Nucleotide analogs (NAs) are a key antiviral class of molecules used to fight HIV and HBV chronic infections, infections for which prodrug strategies have been studied extensively. Recently, **Zlatko Janeba**'s team has reported new PDs bearing tyrosine (Tyr) derivatives instead of the phenol moiety present in standard ProTides, such as the FDA-approved tenofovir alafenamide fumarate (TAF). In human lymphocytes, the most efficient Tyr-based PD reached single-digit picomolar EC_{50} values against HIV-1 and nearly 300-fold higher selective index (SI) than TAF. In human hepatocytes, the most efficient PDs exhibited sub-nanomolar EC_{50} values against HBV and up to 26-fold higher SI than TAF. The reason for this outstanding potency was investigated, and it was found that the most efficient Tyr-based PD had higher pH stability, plasma stability, intracellular uptake, and intracellular release of parent tenofovir than TAF. On the other hand, Tyr-based PD also had considerably lower microsomal stability than TAF; this particular weakness is at the heart of future improvement of Tyr-based PD, along with simplification of the masking groups.

6. Session 3: influenza, RSV, and other respiratory viruses

Chaired by Anne Moscona, Marco Vignuzzi, Cybele Garcia, and Brett Hurst.

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

Rebecca Dutch presented three stories on human pneumoviruses (HMPV) with considerations for development of therapeutics. She began by introducing the viral replication centers, termed inclusion bodies (IBs), which are seen across many negative-sense RNA viruses, and the concept of liquid-liquid phase separation. The first story described investigations of HMPV proteins that drive the process of IB formation and identification of the key role of the HMPV P protein. She presented data suggesting that the P protein alone undergoes phase separation in vitro, unlike other viruses, in which both P and N proteins are needed. HMPV P protein serves as a scaffold to help attract other components, and this process includes key initial interactions between the P and N proteins. Rebecca noted how the replication centers may be targeted by therapeutic strategies, mentioning previously reported drug candidates that affect IB structure and processes.

The second story Rebecca presented demonstrated how HMPV infection affects key steps in nucleotide biosynthesis and alters nucleotide levels, potentially regulating viral transcription and replication. Her collaborator, Dr. Rachel Fearns at Boston University, found an inverse relationship between transcription and replication initiation depending on the relative concentrations of GTP and ATP: increases in GTP result in increased transcription, whereas increases in ATP lead to increased replication. Using a GEVAL sensor and a FRET-based sensor, Rebecca’s laboratory examined the abundance of cellular GTP and ATP, respectively, and found statistically significant changes in GTP levels early in infection. Furthermore, they found increases in metabolites that are important for energy production and nucleotide (purine) production. The group continued to study possible drivers altering nucleotide pool levels in infection. IMPDH is a key control point for GTP synthesis. Filament formation (cytoophidia) blocks negative regulation of IMPDH by GTP, resulting in increased GTP levels. Rebecca’s group found that HMPV infection promotes filament formation and that depleting IMPDH inhibits HMPV replication. Altogether, these findings reveal regulation of nucleotide metabolism in infected cells and the potential of antiviral strategies that synergistically block nucleotide pool levels and polymerization.

The third and final story Rebecca described was her group’s work identifying a new means of HMPV spread. The group found that IBs can be directly moved from one cell to another via virus-induced connections between cells. HMPV proteins localize at three distinct structures in late stages of infection in cells. Notably, virus is detected in actin-based extensions, modifications of actin cytoskeletons that run from cell to cell during infection. These extensions allow direct transfer of inclusion bodies, facilitating cell-to-cell spread of HMPV and other negative-sense RNA viruses.

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

Sean Whelan discussed the development and validation of a chimeric vesicular stomatitis virus (VSV), termed VSV-SARS-CoV-2, which depends on SARS-CoV-2 spike protein (S) for infection of cells, as a surrogate for investigating SARS-CoV-2 entry. The VSV platform is a key tool for studying diverse envelope proteins. Sean presented extensive data characterizing the chimeric virus and confirming utility of the system in neutralization assays. Neutralization of human sera from SARS-CoV-2 survivors was evaluated using the chimeric VSV; Sean's group found a strong correlation between neutralizing curves using VSV and those obtained with SARS-CoV-2. Sean also showed how the VSV system could be leveraged to look at cell entry, in particular allowing comparative evaluation of the drivers of membrane fusion versus endocytosis entry pathways. His group did this using two reporter proteins, one on the core (eGFP-P) and another on the exterior of the virus (S protein). Work conducted with collaborators found that most entry events required virus internalization rather than entry directly from the surface, and that the preferred entry mechanism could shift depending on pH levels.

Finally, Sean discussed how the VSV system could be used to anticipate S protein evolution and its consequences for mAbs and vaccines. S genes of Wuhan-1 SARS-CoV-2 strain and subsequent variants, including D614G, B.1.1.7, BA.1, BA.2, BA.5, BQ.1.1, and XBB.1, were engineered into the VSV genome in place of the endogenous glycoprotein gene. Using panels of neutralizing monoclonal antibodies, multivalent minibinders, and soluble receptor decoys, including those in clinical use or development, Sean's group selected chimeric VSV variants resistant to inhibition. This work yielded a vast library of escape variants that identified critical positions in S associated with resistance to specific inhibitors and established fitness of different S mutations. This knowledge of escape mutants, coupled with sequence analysis of viruses circulating in humans, is critical to inform use of licensed therapeutic antibodies. One notable finding was that some substitutions led to altered receptor usage; for example, E484D resulted in virus capable of infecting cells in an ACE2-independent manner. Additionally, when the group used monoclonal antibodies isolated from individuals vaccinated against new variants and examined escape mutants, they found that selective pressure was driving the virus back to ancestral sequences. This raised the question of whether vaccine-mediated selective pressure can be used to force the virus back to a state that is sensitive to antibodies to which it had previously evolved resistance.

Altogether, Sean showed a breadth of data supporting his group's development of a VSV-based BSL-2 tool that is key to probing the functions of SARS-CoV-2's S protein, namely immunogenicity, antigenicity, cell entry, and envelope protein evolution.

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

Xavier Saelens discussed his group's work with single-domain antibodies, which are very versatile tools for studying and interfering with viral entry pathways. Xavier first

described the group's work with influenza A virus, work that attempted to target the conserved M2 surface antigen with a functionalized single domain antibody (VHH). This was accomplished using a soluble M2e-specific nanobody that, when tested in mice, could confer improved outcomes upon infection. They also found that this antibody could be delivered as an RNA molecule, further improving outcomes in mice. He then briefly discussed their work with pre-fusion, F protein-specific VHHs with strong neutralizing activity against RSV. The group developed two candidates (F-VHH-4 and F-VHH-L66) that neutralized RSV very potently; both were found to bind and stabilize the pre-fusion conformation of the F protein.

To further support use of these antibodies, Xavier highlighted how a SARS-CoV-2 receptor-binding domain- (RBD) specific single-domain antibody was rapidly engineered into a stable anti-COVID-19 biological with excellent manufacturability. When the pandemic began, the group already had a nanobody that could work against SARS-CoV-2. Within two months, they developed an RBD-targeted nanobody, VHH72, which can access its target epitope when at least one RBD trimer is in the up position. Humanizing nanobody 72, incorporating an S56A mutation and fusion with a human IgG1 Fc domain, increased its efficacy, and the product was highly soluble and thus clinically developable. Xavier then discussed newly discovered single-domain antibodies with broad virus-neutralizing activity directed against the spike (S) protein of SARS-CoV-2. When the omicron variant of SARS-CoV-2 arose, many mAbs could not neutralize it in vitro, which was also a problem for VHH72. Since the S2 domain of the SARS-CoV-2 spike protein is more conserved, the group pivoted to targeting this domain with high-potency VHHs and developed S2-specific nanobodies with very potent neutralization. These VHHs do not compete with virus attachment or ACE2 binding; instead, the S2-binding VHHs inhibit post-infection syncytia formation. Escape mutant selection supported an epitope in heptad repeat 2 (HR2), and the group found that the S2-binding VHH recognizes a quaternary epitope in HR2. They developed an Fc-humanized nanobody that neutralizes SARS-CoV-2 and is clinically developable. VHH-Fc fusions were found to neutralize authentic SARS-CoV-2 and were broadly neutralizing against its variants. The mechanism by which these fusion molecules neutralize is thought to include interference with structural rearrangements of the HR2 in the S2 domain, such as initial opening of HR2, that are essential for spike-mediated membrane fusion.

Overall, these single-domain antibody-based biologicals represent much needed drug candidates to prevent and treat disease caused by influenza, RSV, SARS-CoV-2, and other respiratory viruses, particularly for immune-compromised populations with low vaccine responses. The group's findings also highlight that single-domain antibodies are promising candidate therapeutics to control respiratory viruses by targeting unusual epitopes.

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

Manon Laporte opened the influenza, RSV, and other respiratory viruses section of lectures with discussing a novel inhibitor series for SARS-CoV-2 (identified and developed by the EU consortia SCORE and CARE) that targets the membrane protein. A high-throughput

screening assay utilizing VeroE6-GFP cells was used to identify compounds from a small molecule library capable of inhibiting SARS-CoV-2. Antiviral assays confirmed low μM activity of the compound in VeroE6 and A549-ACE2-TMPRSS2 cells. Analogs of the compound identified from the screening assay produced several compounds with double-digit nanomolar activity in both previously mentioned cell lines. The compounds retained activity against SARS-CoV-2 variants. Similar activity was observed against SARS-CoV but activity was greatly reduced against MERS-CoV and hCoV-229E. In human primary airway epithelial cells, a 1.0 μM concentration of two analogs completely inhibited viral replication. In a SCID mouse model of SARS-CoV-2 infection, once or twice daily treatment with CIM-834 at 100 mg/kg produced a similar reduction in virus titers in the lungs as a twice-daily 300 mg/kg dose of nirmatrelvir, used as a positive control.

To investigate the mechanism of action, the SARS-CoV-2 alpha variant was serially passaged with increasing concentrations of these compound. After 20 serial passages, the resulting virus was sequenced and non-synonymous mutations were found in the M-gene. Phenotypic assays demonstrated that the mutated virus was resistant to the analogs of their lead compound but retained activity against remdesivir. The resistance selection studies demonstrated mutations in the M-protein in regions recently shown to interact with the N protein. In vitro replication studies using a SARS-CoV-2 reporter assay indicate that their lead compound and analogs did not inhibit early replication of SARS-CoV-2, supporting a late mechanism of action. These data agree with a mechanism of action related to the budding of the virus. This is a newly identified antiviral target that mutates slowly and is one of the most abundant viral proteins. These findings suggests that the membrane protein may be a promising antiviral target for the treatment of COVID-19.

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

Ruchi Yadav, a Ph.D. candidate, provided background on polyproteins to understand how they can be used for antiviral development. She focused on the translation of the SARS-CoV-2 genome into polyproteins pp1a and pp1ab. She further explained that processing of the Nsp7–11 region of the polyprotein has been identified as a critical step in SARS-CoV-2 replication. This region is first cleaved between Nsp9 and Nsp10, followed by simultaneous cleavage of the Nsp8–9 and Nsp10–11 junctions, with the final cleavage occurring at the Nsp7–8 junction. A similar cleavage order was observed in SARS-CoV but not when only the peptides of junction sites were evaluated. Taken together, these data indicate the polyprotein conformation and structural environment of the cleavage junctions are responsible for determining the order of polyprotein cleavage.

An integrative modeling approach was used to determine possible structural models for the Nsp7–11 polyprotein region. The Nsp7–8 junction, which is incompletely processed in vitro even after 24 h of exposure to M^{pro}, adopts an alpha-helical conformation in most of the models. Its functional role in the viral lifecycle is unknown, but disrupting the Nsp7–8 junction site in coronaviral reverse genetics systems is lethal, suggesting a unique drug target site. The precedent of bevirimat, a potent HIV maturation inhibitor that inhibits the cleavage

event at the CA/SP1 junction of the Gag polyprotein, validates this concept. Since M^{pro} is responsible for cleaving Nsp7–8, Ruchi's group evaluated inhibitors that bind to the M^{pro} active site that interacts with the polyprotein substrate. While nirmatrelvir strongly inhibited M^{pro} processing, none of the allosteric M^{pro} binders found previously by crystallographic screening were able to effectively inhibit M^{pro} processing of the Nsp7–11 polyprotein substrate. These data suggest that allosteric inhibition of M^{pro} may be difficult and may only be efficiently achieved by interface binders destabilizing the M^{pro} dimer.

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

Qi Huang presented on the evolving story of bemnifosbuvir (BEM) as a potential therapeutic for treating SARS-CoV-2 infections. The compound is a guanosine nucleotide prodrug that targets RdRP and could be broadly applicable to future threats and pandemic preparedness. BEM was originally developed as an orally bioavailable drug candidate for treating HCV infection and has maintained an excellent safety profile through clinical trials. During the early stage of the COVID-19 pandemic, BEM was evaluated and found to have sub-micromolar to single-digit micromolar activity against SARS-CoV-2, including variants of concern (VOC), in human tracheal/bronchial epithelial cell culture systems. It was also active against SARS-CoV, human coronavirus 229E (HCoV-229E), and human coronavirus OC43 (HCoV-OC43) in Huh7 cells. MERS-CoV was the only coronavirus that was not sensitive to BEM. Qi described efforts to select for drug resistance to BEM using HCoV-229E. Passaging HCoV-229E in the presence of increasing concentrations of BEM did not result in mutations in the proximity of the catalytic site or the NiRAN domain. Resistance mutations that weakly to modestly increased EC_{50} (7.6- to 8.3-fold) were identified in Nsp12, a region that is not conserved in SARS-CoV-2 and is unlikely to affect nucleotide or nucleotide analog binding. Qi also presented data indicating that remdesivir resistance does not confer cross-resistance to BEM. Thus, early studies suggest that BEM poses a high genetic barrier to resistance to a surrogate coronavirus in cell culture, but it remains to be seen whether SARS-CoV-2 drug resistance will emerge in the clinical setting.

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

A major challenge confronting nucleotide analogs that target the SARS-CoV-2 RdRP is the exonuclease (ExoN) proofreading activity that removes the incorporated triphosphate forms of these antiviral drugs, thereby reducing their efficacy. **Carolina Sacramento** described efforts to target the ExoN activity with small molecule inhibitors previously pursued as HCV inhibitors as a means to enhance the activity of RdRP antivirals approved or being considered as COVID-19 therapeutics. Computational docking studies identified several potential ExoN inhibitors which were tested in ExoN cleavage activity assays; pibrentasvir (PIB) was the most effective. The antiviral activity of PIB and other in vitro ExoN inhibitors was confirmed in Calu-3 cells with dose-responsive inhibition of SARS-

CoV-2 replication. Combinations of PIB or other HCV protease inhibitors with activity against EcoN (ombitasvir, daclatasvir) with clinically approved RdRP inhibitors remdesivir, favipiravir, sofosbuvir, and tenofovir were found to be synergistic in Calu-3 SARS-CoV-2 infection assays. Notably, adding PIB improved the efficacy of remdesivir and favipiravir by 6-fold and 10-fold, respectively. These results suggest that co-administering clinically active ExoN inhibitors may enhance the effectiveness of currently approved RdRP antiviral drugs for treating COVID-19. This rational approach could also improve the activity of nucleotide analogs against other viral infections in which exonuclease-driven proofreading activity limits the efficacy of RdRP inhibitors.

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

Michael Rhodin presented findings on EDP-235, a SARS-CoV-2 3C-like protease (3CLpro) inhibitor being developed by Enanta to treat COVID-19. The compound displays low-nanomolar potency in biochemical assays and in Vero cell culture against infection with SARS-CoV-2 and its variants of concern, including omicron. Other notable preclinical findings include a high barrier to resistance and excellent oral bioavailability. In humans, the compound has an extended half-life of 13–22 h with favorable lung tissue distribution. To assess preclinical efficacy, EDP-235 was evaluated in a ferret model of SARS-CoV-2 replication and transmission in collaboration with Dr. Richard Plemper at Georgia State University. Pharmacokinetic (PK) data in ferrets were consistent with values observed in the human Phase I study. A dose of 200 mg/kg was based on the PK data and administered orally, once or twice daily. Treatments were initiated 12 h post infection (p.i.) with 10^5 plaque forming units (pfu) of SARS-CoV-2 and the efficacy readouts were viral loads in nasal lavages, terminal nasal turbinate tissue viral burdens, and transmissibility to treatment-naïve, uninfected animals cohoused with the various treatment cohorts starting 2.5 days p.i. Twelve hours after initial EDP-235 treatments, infectious viral loads dropped consistently and were largely undetectable by 2.5 days p.i. In contrast to the robust SARS-CoV-2 transmission observed in vehicle-treated ferrets, there was no evidence of transmission by animals treated with EDP-235. Eight days p.i., end-of-study nasal turbinate data found high viral loads only in the ferrets that received the vehicle, with the virus either absent or below detection levels in the animals treated with EDP-235. No differences in efficacy were seen between once and twice daily administration of EDP-235, and viral RNA data for all readouts were consistent with the infectious virus assay findings. The data presented showed a rapid antiviral response in the lungs of infected ferrets treated with EDP-235, with the potential of the treatment to lessen or prevent household or close-contact transmission. EDP-235 is currently in Phase II clinical trials, with the results expected in May 2023.

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

The initiation of infection by coronaviruses is mediated through the interaction of the spike glycoprotein with the host cell receptor, facilitating membrane fusion and viral entry. **Jing Jin** described structural and glycomics studies of the native human coronavirus NL63 (HCoV-NL63) spike glycoprotein. The group determined site-specific glycan composition

and occupancy using mass spectrometry and built a full-length, fully glycosylated spike structure model using high-resolution cryogenic electron tomography (cryoET) coupled with subtomogram averaging and molecular dynamic simulations. The model revealed an oligomannose glycan shield on HCoV-NL63 spike surface that correlates with the strong immune evasion of HCoV-NL63 and its lack of adaptive evolution. With these integrated analyses, the group elucidated the conformational landscape of the spike with the compact crown bending up to 80 degrees of freedom relative to the stalk that anchors the spike to the surface of the virion. N1242-linked glycan at the hinge region connecting the spike crown with the stalk plays a critical role in regulating the extensive orientational freedom of the crown and epitope shielding of a potential neutralizing epitope. Notably, functional assays using pseudotyped viruses lacking this N1242-specific glycosylation showed dramatically reduced infectivity. Although Jing's presentation did not include specific antiviral compounds and data, it served to provide insight and a fresh perspective on the dynamics of coronavirus spike proteins with applicability to the design of future vaccines and antiviral strategies that interfere with the initial step of infection by pathogenic coronaviruses.

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

Influenza A viruses (IAVs) pose a continual threat to public health due to their ability to rapidly evolve to evade natural and vaccine-induced immunity and due to their increasing resistance to approved antiviral drugs. In her presentation, **Jazmin Galván Achi** from Dr. Lijun Rong's lab (UIC) describes the lab's efforts to identify novel small molecule entry inhibitors targeting the hemagglutinin (HA) of group 1 (H1N1 and H5N1) and group 2 (H3N2 and H7N9) IAVs. In high-throughput screening (HTS) studies performed by the lab, retroviruses pseudotyped with group 1 or group 2 HA were used to test 19,200 and 10,000 compound libraries, respectively, for hits with inhibitory activity specific for IAV entry. The best hit from the group 2 HTS was CBS1194, a compound with 250 nM activity against pseudotyped H7N1 virus and 1.6 μM activity against infectious H3N2 virus. Structure-activity relationship studies led to the synthesis of a derivative compound, 4L, which showed increased potency of 40 and 30 nM against pseudotyped H7N1 and infectious H3N2 viruses, respectively. Molecular docking and mutagenesis studies revealed that 4L interacts with the stalk region of H7. For group 1 IAVs, compound CBS1117 was the most exciting hit, with low micromolar activity against pseudotyped H5N1 virus and 270 nM activity against infectious H1N1. Crystallographic data identified the binding region of CBS1117, leading to the rational design of ING-1466, a derivative with potency of 12.6- to 3.9-fold higher against pseudotyped H5N1 and infectious H1N1 viruses, respectively. ING-1466 was then advanced into mouse efficacy studies in a PR8 H1N1 intranasal challenge model. Lung replication was measured and ING-1466 was found to have activity comparable to the positive control oseltamivir when given once daily at 50 or 25 mg/kg by oral gavage. Further, combination therapy with suboptimal doses of ING-1466 and oseltamivir resulted in complete protection against a lethal challenge dose of PR8. These findings highlight the discovery and development of promising new small molecule inhibitors targeting IAV entry, with the potential to synergize with existing first-line antivirals such as oseltamivir.

7. Session 4: late-breaking oral presentations

Chaired by Robert Jordan and Angela Corona.

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

Herpes simplex virus (HSV) establishes life-long infections characterized by periodic reactivation. HSV-1 and HSV-2 infect epithelial cells in the oral and genital mucosa, causing skin lesions that resolve but can reoccur upon virus reactivation. HSV-1 and 2 can be detected in neurons of the sensory ganglia, the primary site of latent infection. Nucleoside analogs like valacyclovir, acyclovir, and famciclovir have been used to treat HSV-associated lesions but do not impact the latent reservoir or frequency of reactivation in patients. New therapies that can reduce reactivation frequency will significantly impact patient care.

Gerald Kleymann reported on IM-250, an HSV helicase primase inhibitor with activity against HSV-1 and 2. It was evaluated in mouse and guinea pig models of HSV-1 and HSV-2 infection, respectively, and found to reduce disease during acute infection and also to decrease reactivation when administered as an intermittent therapy over a period of time. IM-250 was administered to mice at 5 mg/kg/day and to guinea pigs at 5–15 mg/kg/day. Mice were inoculated with HSV-1 (17VP16placZ) by the ocular route; once latency was established, virus could be reactivated by heat stress. Guinea pigs were inoculated intravaginally to establish a latent infection that spontaneously reactivates, causing lesions and virus shedding. Treatments were administered orally, with acyclovir control provided in water and IM-250 mixed in with chow. Four cycles of intermittent IM-250 administration were sufficient to reduce the number of neurons undergoing reactivation in mice compared to placebo treatment. In guinea pigs, 6 months of intermittent therapy with IM-250 decreased the frequency of reactivation by 77%, with complete inhibition of recurrent disease after 7 cycles of therapy. The number of neurons that reactivate following explant of sensory ganglia was significantly reduced in the IM-250 treatment group. Thus, IM-250 decreases the pool of reactivatable virus after intermittent treatment. This is the first indication that an antiviral compound can impact the latent HSV reservoir.

7.2. Intranasal Delivery of Fusion Inhibitory Lipopeptides Blocks SARS-CoV-2-Induced Pathology in Mice and Permits Establishment of Long-Lasting Protective Immunity. Branka Horvat, M.D., Ph.D., International Center for Infectiology Research (CIRI), Lyon, France

Novel lipid peptides that target heptad repeat regions of viral fusion proteins have been shown to inhibit viral entry by interfering with the structural reorganization of the fusion protein required to facilitate viral entry, and consequently prevent the transmission of SARS-CoV-2 in ferrets. **Branka Horvat**'s presentation demonstrated that fusion inhibitory peptides inhibit SARS-CoV-2 infection in organotypic cell cultures and in a ACE2 (K18-hACE2) murine model of SARS-CoV-2 infection when delivered intranasally at 4 mg/kg per day during the first 3 days of infection. Mice receiving the lipid peptides and infected with SARS-CoV-2 maintained body weight and showed greatly reduced virus shedding and mortality. In addition, the group observed decreased viral loads and normalization of

the transcriptomic profile in lungs, including the expression of both innate and adaptive immunity gene clusters, which could impact disease progression. Finally, mice treated with lipid peptides and infected with SARS-CoV-2 developed protective immunity and were completely resistant to a second challenge with a lethal dose of SARS-CoV-2. These results suggest a novel way of protecting from disease while also allowing development of protective immunity, which could be further utilized against SARS-CoV-2 and the other airborne viruses.

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

Rintatolimod is a synthetic derivative of inosinic acid with antiretroviral and immunomodulatory properties approved for treatment of chronic fatigue syndrome. Rintatolimod stimulates the innate immune system by binding to and activating toll-like receptors 3 (TLR-3). **Angela Corona** discussed a study in which the antiviral activity of rintatolimod was evaluated in a mouse model of Ebola virus infection and the mechanism of action was studied in cell-based and biochemical assays.

BALB/c mice were infected with 1000 pfu of Ebola virus (Zaire) and treated with vehicle or with 6, 12, or 18 mg/kg rintatolimod administered by intraperitoneal injection. Rintatolimod conferred >90% protection from lethal disease at all doses compared to vehicle-treated animals, which succumbed to infection by day 7. Rintatolimod efficiently competed with dsRNA for VP35 binding, suggesting that the compound blocks VP35-mediated inhibition of the interferon response. Consistent with this hypothesis, IFN production was restored in cells expressing VP35 and transfected with dsRNA derived from influenza virus to stimulate the interferon response. This work supports further investigation of rintatolimod as a treatment for Ebola virus infection.

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

Repurposing drugs for development of antiviral preparations requires that compounds be active against the virus at a suitable dosing regimen that is safe and effective. It also requires that drugs that are typically highly optimized for one molecular target in one organ or tissue are active against another molecular target in a different organ or tissue. To provide an alternative to directly repurposing drugs, **Consuelo Correa-Sierra**'s group evaluated mefloquine analogs in cell-based assays of CoV-OC43 replication. The study identified compounds that inhibited virus replication with limited cytotoxicity; these will serve as starting points for compound optimization to improve efficacy and antiviral properties. The selected compounds had similar antiviral activity against SARS-CoV-2 and, surprisingly, CMV replication. Using these scaffolds as starting points, the investigators plan to optimize these compounds using medicinal chemistry to identify candidates that can be advanced as broad-spectrum antivirals. While deconstructing hits identified by screening repurposed compound libraries may not provide a rapid path to a clinical candidate, these drugs

can serve as starting points for drug discovery programs making use of already available medicinal chemistry information.

8. Session 5: chronic, persistent, or latent viruses II

Chaired by Antoine Alam, Graciela Andrei, Chris Meier, and Jennifer Moffat.

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

The session on Chronic, Persistent, or Latent Viruses II on Thursday morning, chaired by Antoine Alam and Graciela Andrei, focused on herpesviruses and HIV.

The session opened with a presentation by **Jocelyne Piret** discussing the clinical challenges in HCMV therapy and the urgent and unmet need for new anti-HCMV antivirals. Jocelyne started her talk with an overview of primary, reactivating, and super HCMV infections. In most healthy patients, HCMV infections result in only mild flu-like febrile illness. However, this virus presents a major challenge in post-transplant conditions, resulting in life-threatening tissue-invasive disease (most commonly in the lung, GI tract, liver, eye, or CNS) and greatly increasing the risk of graft loss and mortality. Current diagnostics are highly sensitive and high throughput, although they can be challenged by viral compartmentalization within anatomical sites in the absence of viremia. Immune monitoring, mostly by IFN-γ-producing cells, can be useful to predict risk of HCMV rebound, monitor prophylaxis efficacy, or monitor the potential for relapses after treatment; however, this needs further investigations.

Until recently, HCMV treatment was restricted to ganciclovir (GCV) and valganciclovir (VGCV) for first-line treatment and foscarnet (Fos) and cidofovir (CDV) as second line. The acyclic nucleosides GCV and VGCV are activated by the UL97 viral kinase and then by cellular kinases, whereas CDV is activated by cellular kinases only and Fos requires no activation. Preventing infection is based on universal prophylaxis, administering an antiviral agent for 3 or 6 months after transplant. Pre-emptive therapy consists of monitoring viral loads and initiating treatment when these loads increase above certain pre-determined levels. The first line of treatment is oral VGCV or intravenous GCV, the latter preferred for life-threatening disease. Treatment is stopped upon clinical or virological response. Resistance should be suspected when there is no response to treatment. Risk factors differ in solid organ transplant or hemopoietic stem cell transplant (HSC) patients but are associated with prolonged antiviral drug exposure or suboptimal blood drug levels.

Resistance to GCV and VGCV is normally below 5% but increases to above 10–15% in recipients of lungs and in haploidentical grafts in HSC recipients. Early resistance maps to the UL97 kinase, largely clustered around position 590–607. Some mutations confer only moderate levels of resistance (<5-fold), whereas other result in high levels of resistance. Later resistance maps to the UL54 polymerase, and combined mutations in both kinase genes result in high levels of resistance (15–30-fold). Mutations in UL54 often confer resistance to more than one polymerase inhibitor: GCV/CDV, GCV/Fos, or GCV/CDV/Fos.

CDV and Fos require intravenous administration, are limited by nephrotoxicity, and often result in sub-optimal outcomes, and GCV is myelotoxic. New antivirals are needed.

Two new antiviral drugs have been recently approved for HCMV treatment: the UL97 inhibitor maribavir (MBV) and the terminase inhibitor letermovir (LMV). LMV (first in class) targets the UL56 subunit with an EC_{50} of 5 nM; it is inactive against other herpesviruses. It has been approved for prophylaxis in recipient HCMV-positive (R^+) allogeneic HSC. The approval was based on a pivotal clinical trial in which clinically significant HCMV infection decreased from 61% with placebo to 37% with LMV at week 24, and overall mortality was reduced from 25% to 21% in the treatment group. Clinical or experimental resistance maps to the UL56, UL89, or UL51 (only two mutations were detected but one was in a clinical patient) subunits of the terminase, with resistance levels varying from as low as 2-fold to as high as almost 9,000-fold, with a low genetic barrier. Mutations in UL89 and UL56 are additive, while those in UL51 and UL56 are synergistic. LMV is safe and well tolerated, has good oral bioavailability, and poses no risk of cross-resistance with other HCMV drugs, but it affects cytochrome p450 and transporters, so drug interactions are highly expected. Two recent clinical trials show activity, but its use is mostly as prophylaxis. Because of its low genetic barrier, LMV is not being evaluated as treatment option.

Jocelyne discussed several recent or ongoing clinical trials with MBV, which targets the UL97 kinase and is approved for treating adult and pediatric patients with post-transplantation HCMV disease refractory or resistant to polymerase inhibitors. The approval was based on a pivotal clinical trial showing that, after 8 weeks, MBV increased HCMV clearance from 23.9% to 55.7%; at 9–16 weeks, the increase was from 10% to 19%. Moreover, clinically relevant recurrences decreased from 36% to 26% in responders, although mortality was similar between arms. Resistance mapped to the UL97 kinase in the vicinity of the active site and can confer cross-resistance to GCV. Resistance easily reaches more than 100-fold. Compensatory mutations in UL27 are selected and further increase resistance by ~2-fold. MBV is safe and well tolerated, with low myelotoxicity and good oral availability, but it is limited by co-resistance with GCV and its genetic barrier to resistance may be low. It also has poor eye and CNS penetration.

Both MBV and LMV have some interactions with other antivirals. MBV shows antagonistic effects with GCV, but is synergistic with Fos, CDV, and LMV. LMV is additive or moderately synergistic with all other drugs except Fos, with which it is mildly antagonistic.

No guidelines yet exist for using LMV or MBV. LMV may be used for prophylaxis only under certain conditions like myelosuppression or in adult R^+ allo-HSC recipients. MBV is an option for treating refractory/resistant CMV infection, mild and moderate disease, and renal dysfunction or myelosuppression.

Jocelyne’s talk thus highlighted the still ongoing struggles with HCMV therapy in transplant patients and the yet unsatisfied need for well-tolerated antivirals with high genetic barrier to resistance, no myelosuppression, and good penetration to the CNS and eye. Her talk

provided the direct link between the developing novel antivirals and the clinical needs of patients.

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

The second talk of the session was by **Benjamin (Ben) Berkhout**. His talk started with a discussion about the potential of using CRISPR/Cas9 against HIV. CRISPR/Cas could make T-cells resistant to HIV and cure them of integrated virus, neither of which was possible before. However, many surprises were also found along the way, including escape mutants. These mutants had deletions at the cleavage site, preserving a type of mutation that had been only rarely observed in HIV evolution studies. These indels are not unexpected from CRISPR/Cas, as they result from the non-homologous DNA repair mechanism, but they end up helping the virus escape treatment. A bigger surprise was finding that targeting two sites at the same time with two different gRNAs seldom resulted in large deletions between the two cut sites; rather, the predominant mutations were indels at both positions. Ben's group concluded that DNA repair is too fast and thus each individual cleavage is repaired before the other one is introduced. A worse surprise was that many large deletions are often seen, expanding well beyond the integrated pro-virus. In brief, the potential use of CRISPR/Cas to cure or prevent HIV needs to consider this spectrum of effects, including the potential for escape mutants and larger mutations extending into the host's genome.

Ben next discussed how to target latently HIV-infected cells. Some reports had suggested that most of the latent reservoir is in CD32a CD4 T cells. Although this model has been disputed, Ben's group has reproduced these data after many years. These T cells are less than 0.2% of the total but are 1,000-fold enriched in HIV DNA, opening the possibility of targeting them to cure latency. His group has selected darpins as a mechanism to direct agents directly and specifically to this CD32a-positive population.

Ben then moved to the main thrust of this talk: analyses of resistance to dolutegravir, an integrase inhibitor. He thanked Vincenzo for his timely introduction to HIV integration and introduced the polypurine track (PPT) in HIV and its role during reverse transcription, which is priming the synthesis of the second DNA strand. He then briefly summarized the mechanism of action of dolutegravir, with an emphasis on its binding to both the integrase complex and the viral cDNA. The PPT, highly enriched in purines in the RNA strand and pyrimidines in the DNA strand, is resistant to RNaseH and thus serves as a primer for synthesis of the second DNA strand at the LTR, which eventually produces the 2-LTR linear proviral DNA that then integrates into the host genome. Dolutegravir has a high genetic barrier to resistance and is thus a preferred drug in antiretroviral combination therapy. Resistance is difficult to select for, and when it is selected, the mutations map to the PPT (which overlaps Nef) rather than the sequences coding integrase. One dolutegravir resistance mutation was selected in culture and another was isolated from a patient who was no longer responding to treatment. As Nef is not required for viral replication in culture, resistance in culture cannot result from Nef mutations. Ben's group generated a library of HIV genomes with randomized PPT. In the absence of any drug, the wild-type PPT sequence soon outgrew all other sequences. However, the mixed sequences persisted when the mixed population

was grown in the presence of dolutegravir, indicating that many different PPT mutations can confer resistance. All these mutations drastically decrease HIV fitness. Molecular analyses indicate that these PPT mutations block HIV integration and increase extrachromosomal 1-LTR circular HIV episomes. Most importantly, all PPT mutants are resistant to dolutegravir; consequently, mutations that disrupt integrase activity (DDE to NNQ) have no phenotype while severely impairing replication of wild-type virus and preventing its integration. Part of this work has been published in *Antimicrobial Agents and Chemotherapy*, and the bulk has recently been accepted for publication in the *Journal of Virology*; the Delelis lab in Paris found similar results. This is a novel resistance mechanism, which highlights once again the flexibility of viral genetics, as discussed in Dr. Domingo's plenary talk at the opening session. PPT mutants should be resistant to all integrase inhibitors. The advice would be to screen patients who fail to respond to dolutegravir for PPT mutations. As a closing corollary, the mutant behaves basically like HBV, raising the exciting prospect of a potential evolutionary path between retroviruses and hepadnaviruses. Ben's talk thus covered potential pitfalls of CRISPR/Cas therapy for HIV, raised the prospect of targeting the population of latently infected cells in HIV patients, and uncovered a new resistance mechanism against integrase inhibitors. Fortunately, the resistant viruses show low fitness and may not pose a major clinical challenge to the use of integrase inhibitors.

8.3. Combining Autofluorescent ANCHOR-Tagged Viruses with High-Content Imaging for the Discovery of New Broad-Spectrum Herpes Virus Inhibitors. Franck Gallardo, Ph.D., NeoVirTech SAS, Toulouse, France

Herpesviruses are a family of large dsDNA viruses, including HSV-1 and 2, CMV, and varicella zoster virus, which infect people worldwide. Although primary infection is often silent, herpesviruses remain in a latent form that can reactivate to trigger potentially life-threatening recurrences. Several antivirals have been developed, including DNA polymerase inhibitors (acyclovir, ganciclovir), terminase inhibitors (letermovir), and primase inhibitors (pritelivir). As repeated treatment with an antiviral can select for resistant strains, causing therapeutic failure, there is a constant need for the development of new antiviral therapies.

To reach this goal, **Franck Gallardo**'s group developed a collection of autofluorescent ANCHOR-tagged viruses and used high-content imaging techniques, allowing visualization of virus infection, replication, and propagation in living cells in the presence of a compound of interest. These viruses are extremely useful to decipher and understand the mechanism of action of new antiviral compounds.

Over the past years, Franck's group developed and optimized a collection of acyclonucleoside phosphonate molecules that inhibit herpes virus replication in vitro. LAVR-289 was selected as a lead compound. LAVR-289 is designed as a prodrug with three biolabile groups that must be cleaved by cellular enzymes to liberate the active form. LAVR-289 abolished HCMV virus replication at nanomolar concentrations, being at least 50 times more potent than other nucleoside inhibitors. High-resolution microscopy showed that LAVR-289 blocks HCMV replication at the early replication center stage, indicating that it prevents viral DNA replication. LAVR-289 was active against HCMV strains resistant to approved antiviral drugs, such as ganciclovir and letermovir, and in combination with

these displayed synergistic and antagonist interactions, respectively. Broad-spectrum anti-herpesvirus activity of LAVR-289 was detected using a large collection of herpesviruses (13) that impact human and veterinary health. LAVR-289 prevented HCMV replication in human placenta villi ex vivo and varicella replication in a humanized mouse model when administered either subcutaneously or orally. Interestingly, LAVR-289 inhibits replication of all dsDNA viruses tested so far, including the Mpox strain circulating in 2022, with an EC_{50} < 100 nM, by targeting a specific domain in the viral polymerase. Next, LAVR-289 will be challenged in two animal models infected with poxvirus to measure its capacity as a medical countermeasure to poxvirus outbreaks.

8.4. High-Throughput Discovery of Small Molecular Inhibitors of Hepatitis B Virus Subviral Particle Biogenesis. Ju-Tao Guo, Ph.D., Baruch S. Blumberg Institute, Doylestown, Pennsylvania, United States

In addition to releasing infectious HBV virions, HBV-infected hepatocytes also secrete RNA-containing or genome-free virion-like particles, naked capsids, and subviral particles (SVPs). SVPs consist of only small (S), middle (M), and large (L) envelope (surface) proteins and are spherical or filamentous lipoprotein particles with a diameter of 22 nm. In fact, SVPs are the predominant viral product made by HBV-infected hepatocytes and exceed the virion particles in the blood of HBV carriers by 10,000- to 100,000-fold. Although the function of SVPs in HBV infection remains elusive, the high levels of HBV surface antigen (HBsAg) seen in SVPs in the blood of chronic HBV carriers is considered to drive the exhaustion of antigen-specific T and B lymphocytes to allow persistence of viral infection. Accordingly, therapeutic reduction, and ideally elimination, of HBsAg should facilitate the recovery of host adaptive antiviral immune responses and functional cure of chronic hepatitis B. Although siRNA therapeutics can reduce HBsAg load by approximately 100-fold, sero-clearance of HBsAg apparently requires additional antiviral drugs, preferably acting via distinct mechanisms. Recently, the group discovered that an amphipathic alpha helix at the C-terminal region of the antigenic loop of S envelope protein plays an essential role in S protein oligomerization and morphogenesis of HBV SVPs, and disrupting the structure of the alpha helix results in S degradation by 20S proteasomes. **Ju-Tao Guo**'s group thus hypothesized that pharmacologically disrupting SVP morphogenesis by specifically targeting S protein oligomerization and SVP budding will reduce HBsAg. To discover small molecule compounds with such antiviral properties, they established a cell-based assay for high-throughput screening of compound libraries and assays for selecting screening hits that specifically inhibit SVP production. Their pilot screen campaign with a bioactive compound library identified inhibitors of valosin-containing protein (VCP)/p97, E3 ubiquitin ligase MDM2, and 26S proteasomes; these compounds significantly reduced intracellular production of SVPs and the function of those cellular proteins in SVP biogenesis had been further validated by siRNA knockdown. HBsAg secretion inhibitors identified from this high-throughput screen of 26,900 compounds are currently under characterization.

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

With nearly 300 million individuals chronically infected, HBV represents a serious global health problem. Currently available treatments do not prevent HBV rebound from viral genomic reservoirs, namely the covalent closed circular DNA (cccDNA). Also, these treatments do not silence the expression of HBsAg, produced in part from the integrated HBV DNA. Therefore, there is an unmet need for developing novel therapeutics targeting HBV cccDNA and integrated DNA.

In this report, **Anuj Kumar**'s group aimed to target HBV cccDNA and integrated viral DNA using cytosine base editors (CBEs). CBEs enable precise and permanent conversion of cytosine into thymine within DNA without generating double-strand breaks. A base-editing strategy was devised to introduce stop codons in HBV genes *HBs* and *Precore* using two distinct gRNAs, named gS and gPC, respectively. The antiviral efficacy of this approach was first assessed in a HepG2-NTCP cell line and primary human hepatocytes (PHHs). Transfection with CBE mRNA and the combination gS + gPC enabled robust cccDNA editing and sustained inhibition of HBV parameters, including HBsAg, HBeAg, 3.5 kb viral RNA, and total intracellular HBV DNA. This antiviral effect was observed both as a single treatment and in combination with a nucleoside analogue. Importantly, gS + gPC prevented viral rebound compared to nucleoside analog monotherapy in HBV-infected PHHs. Further, Anuj's group investigated the effect of HBs targeting gS and gS + gPC in HepG2.2.15 cells, which harbor artificially integrated HBV DNA. They observed remarkable suppression of HBsAg. Similar observations were seen in PLC/PRF/5 cells containing naturally integrated partial HBV DNA sequences.

The gS + gPC combination was also evaluated in the HBV minicircle mouse model system. This model allows HBV replication and viral antigen expression resulting from hydrodynamic injection with a recombinant cccDNA-like plasmid. After the establishment of HBV replication, hepatocellular delivery of the base-editing reagents was achieved via systemic administration of lipid nanoparticles (LNP) formulated with CBE mRNA and gS + gPC. Intravenous injection of this LNP formulation resulted in significant reduction of HBV DNA, HBsAg, and HBeAg in mouse serum. Altogether, the data demonstrate that cytosine base editing of HBV cccDNA and integrated DNA potently suppresses viral replication and HBsAg expression.

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

Hepatitis E virus (HEV) is a leading cause of viral hepatitis, causing ~20 million new infections each year, of which ~3.3 million become symptomatic. It is fecal-orally transmitted through contaminated drinking water or undercooked meat. While HEV variants causing human infection predominantly belong to the *Orthohepevirus* species A (HEV-A), several human infections with rat hepatitis E virus (*Orthohepevirus C* species; HEV-C1)

have been reported in recent years. Thus, HEV-C1 should be considered an emerging cause of viral hepatitis in humans. An HEV vaccine (Hecolin) is only available in China and Pakistan; no HEV-specific treatments are available besides off-label use of ribavirin, which is associated with treatment failure and side effects like severe anemia. Ribavirin monotherapy is also effective against HEV-C1 infections in patients, although not all patients are initially responsive to the first course of RBV treatment. Safe and more potent anti-HEV drugs are therefore urgently needed. Previously, **Xin Zhang**'s group reported high susceptibility of athymic nude rats to rat HEV infection when the nude rats were injected intravenously with infectious rat liver homogenate. Here, Xin's group established and standardized an athymic nude rat HEV transmission model and explored the dynamics of HEV replication in rats. Infectious HEV was found in the feces of intravenously infected rats. They showed that the transmission of rat HEV can occur via the fecal-oral route, as rats became positive after oral ingestion of infectious feces. The transmission model was standardized by orally giving the rats a fecal suspension containing pre-defined viral RNA copies. To validate this fecal-oral infection model, the efficacy of ribavirin in lowering viral RNA levels was assessed. Fecal-orally inoculated rats treated once daily with ribavirin (60 mg/kg for 12 days) had significantly lower viral RNA levels (~100-fold less) in feces, liver, and other tissues than did vehicle-treated rats. However, ribavirin did not cure the infection. Altogether, the group proposes that HEV-C1 transmission to humans likely occurs through exposure to infectious rat excrement. The results also showed that the athymic nude rat model is highly suitable for evaluating the efficacy of (novel) antiviral agents or vaccines in blocking or delaying fecal-oral HEV transmission.

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

HIV is still considered an epidemic, affecting 38.4 million people worldwide. HIV-1 is usually treated with antiretroviral therapy comprising ≥2 drugs, including at least one HIV-1 RT inhibitor. Indeed, of the five categories of inhibitors targeting the HIV-1 viral life cycle, ~50% of the FDA-approved drugs target reverse transcription. The drugs that target RT include the nucleoside and non-nucleoside RT inhibitors (NRTI and NNRTI, respectively). NRTI-triphosphates inhibit elongation following binding at the polymerase active site and incorporation into DNA. NNRTI bind an allosteric pocket ~10 Å away from the polymerase active site. Recognizing the proximity of the two drug-binding pockets from the HIV-1 RT three-dimensional structure, in 1993, **Natalie Losada**'s group proposed the idea of making compounds containing both NRTI and NNRTI moieties connected by a suitable linker that could bridge the two sites. Compounds binding in this bridging area have previously been investigated, including in proof-of-concept computational models, using an RNase H inhibitor, and using compounds developed by Merck. This study focuses on compounds (NBD derivatives) originally developed to bind to HIV-1 gp120, of which some were found to inhibit RT. These compounds were developed in Asim Debnath's lab at the New York Blood Center to act as viral entry antagonists. Previously, Natalie's group determined structures of RT with NBD derivatives, observing binding in the vicinity of the primer grip in the palm subdomain, bridging the dNTP- and NNRTI-binding sites. The

lead NBD derivatives have antiviral activity, low toxicity, and RT inhibitory activity (e.g., NBD-14189: EC_{50} = 89 nM, CC_{50} ≈ 21 μM, IC_{50} < 3 μM). Additionally, the crystal structures allowed the group to compare NBD derivatives with other compounds binding RT in the same region, revealing that the NBD compounds had more extensive interactions with conserved residues Asp186, Asp110, and Trp229. In her talk, Natalie reported insights into the potential mechanism of action of NBD derivatives from the structure of RT with bound dsDNA template-primer and NBD-14189. In vitro inhibition studies revealed that NBD compounds can bind in the presence of nucleic acids, so further crystallography studies were performed. Crystal structures of RT in complex with DNA and NBD-14189 (or NBD-14270) showed that the interaction pattern between the NBD derivatives and RT conserved residues is conserved when DNA is also bound. Binding of the NBD compounds is accompanied by displacement of the 3′ end of the DNA primer by ~3 Å away from the polymerase active site (when compared to RT/DNA without NBD; PDB 5D3G). The impact of NBD binding to RT/DNA is reminiscent of that of the FDA-approved non-nucleoside RT inhibitors, albeit targeting a different and more conserved pocket, suggesting an allosteric mechanism of action and a higher genetic barrier to resistance. Additional structural, biochemical, and biophysical investigations of the NBD compounds will help to further elucidate their molecular mechanisms of RT inhibition.

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

The nuclear farnesoid X receptor (FXR) is a master regulator of hepatocyte differentiation and function. Some agonists and ligands of FXR have been shown to inhibit the replication of HBV and HDV propagation. **Romain Barnault** described his group's work using five different FXR agonists with different structures (GW4064, tropifexor, vonafexor, cilofexor, and nidufexor) to further characterized these inhibitory phenotypes in relevant in vitro models (PHH and depart), thus confirming an FXR-dependent class effect.

In its PEGylated form, IFNα is still often used as the first-line treatment in HBV and HDV patients despite its low tolerance and subsequent relative limited efficacy. The combination of PEG-IFNα with vonafexor, a non-steroidal non-bile acid, and highly selective FXR agonist, has been shown in an open-label phase II trial (NCT04365933) to significantly reduce HBsAg levels in HBe-negative HBV-infected patients. This stronger combination inhibitory phenotype has been recapitulated in Romain's models with various FXR agonists against HBV and HDV replication in the absence of any drug toxicity. The inhibition of HBV RNA and HBsAg biogenesis was particularly strong, as well as inhibition of HDV propagation by affecting specific infectivity of secreted particles. Run-on experiments revealed transcriptional downregulation of HBV RNA synthesis. Other mechanisms of action are currently under investigation. Of note, the expression of HBe alone in hepaRG or PHH infection with HBe-negative virus did not change the FXR pathway and ISG induction, indicating that a different parameter correlating with the HBe-status of the patients regulates the effect of the combination in the cohort.

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

Owing to a structural conservation between the corresponding molecular targets at the polymerases active site, currently approved oral treatments for chronic hepatitis B rely on nucleos(t)ide-based drugs originally designed as HIV inhibitors. Lacking a high-resolution structure of the HBV polymerase (HBV pol) and thus the ability to conduct a focused structure-based drug design of HBV pol-specific inhibitors, **Elisabetta Groaz**'s efforts are devoted to gathering crucial structure-activity relationship data that may help design more potent and selective anti-HBV compounds. To this end, different series of nucleoside phosphonates featuring either a 3-fluoro-2-(phosphonomethoxy)propyl (FPMP) or 2-substituted-3-hydroxy-2-(phosphonomethoxy)propyl (2-R-HPMP, where R = Me, C≡N, or C≡CH) pseudosugar moiety connected to natural nucleobases were synthesized along with their phosphonodi- and monoamidate prodrugs. The amino acid at the prodrug moiety was carefully selected to favor first-pass metabolism in the liver, which is highly desirable for targeting liver infection. Effective synthetic routes were developed to generate multi-gram quantities of these compounds in an enantiomerically pure form. Selected analogs were demonstrated to effectively inhibit the replication of HBV, with EC_{50} values in the submicromolar range. Interestingly, their nucleobase specificity was found to vary as a result of different modifications. Antiviral efficacy comparable to that of TAF was observed for a diamylaspartate monoamidate prodrug of (*S*)-FPMPA in a transgenic HBV mouse model. On the other hand, the introduction of a methyl, cyano, or ethynyl substituent at the 2-position of HPMP nucleotides induced a shift in the selectivity spectrum of these compounds from herpesviruses towards HBV. Notably, in the presence of a 3-hydroxyl group, all 2-substituted HPMPs exhibited no or only relatively weak activity against HIV. Phosphonodiamidate prodrugs of (*S*)-methyl- and (*S*)-ethynyl-substituted HPMP guanine-containing analogs exerted remarkably high in vitro anti-HBV activity combined with excellent selectivity, proving to be promising candidates for further anti-HBV drug development. Biochemical and structural studies are underway to elucidate the mode of action of these nucleotide analogs.

9. Session 6: SARS-CoV-2, arboviruses, other biothreat viruses, and broad-spectrum antivirals II

Chaired by Andrea Brancale, Jessica Spengler, Zlatko Janeba, and Christina Spiropoulou.

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

Arenaviruses cause severe human disease, but treatment options are limited. **Allison Groseth** presented on the importance of understanding arenavirus-host interactions to better inform rational design of antiviral strategies. Her lab uses comparative studies between less pathogenic arenaviruses like Tacaribe virus (TCRV) and closely related pathogenic arenaviruses like Junín virus (JUNV) to identify differences in their biology related to

pathogenesis. They and others have observed that TCRV and the attenuated Candid 1 JUNV vaccine strain induce apoptosis in cells, whereas JUNV does not. This observation led to a series of studies into the mechanisms involved in regulating apoptosis in response to arenavirus infection and its consequences for virus biology.

The group identified several pro-apoptotic factors responsible for triggering apoptosis in response to infection, including p53, Puma, and Noxa, as well as anti-apoptotic changes in Bad. Allison then also presented her group's work on the activation of protein kinases (p38, JNK) upstream of these pro-apoptotic factors. Interestingly, while inhibitor studies showed that activation of these kinases is crucial for virus infection, knockout studies of Puma, Noxa, and Bad did not affect TCRV virus replication. However, their activation enhanced the levels of phosphatidylserine (PS) exposure on viral particles and correspondingly increases infection of key target cells (macrophages), suggesting that these changes may, under certain conditions, allow viruses access to cells in the absence of their usual receptors.

In addition to work on host factors within these pathways, Allison discussed studies on the role of arenavirus proteins in their regulation. Her lab observed that while Z expression alone is sufficient to induce apoptosis, the JUNV nucleocapsid protein (NP) can negate Z protein-mediated caspase activation in cell culture, implying a role for NP in actively suppressing apoptosis induction during JUNV infection. Notably, they found that this was related to caspase cleavage of JUNV NP (but not TCRV NP), leading to generation of smaller NP isoforms. When the group used reverse genetics to create viruses without these cleavage sites in NP, they could restore caspase activation, supporting a model wherein pathogenic JUNV actively suppresses caspase activation by sacrificing a subset of its NP as a decoy substrate for caspase cleavage. Importantly, as these smaller isoforms retain accessory functions (antagonism of the IFN response and dsRNA-exonuclease activity) while exhibiting altered intracellular localization, they may have additional roles in regulating the antiviral response to infection.

Allison finished her talk by presenting directly applicable progress in the antiviral field, with recent reports of compounds that target apoptosis regulation and PS, and emphasizing that a better understanding of the virus-host interface during apoptosis regulation may facilitate rational repurposing of available treatments in the future.

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

Drug repurposing, which involves evaluating libraries of drugs already utilized in the clinic for treatment of other diseases, can be useful to rapidly identify active compounds with known profiles and could have the potential to speed up drug development for emerging infectious diseases. **Robert Davey**'s group aims to reduce distraction from off-target effects, use drugs to identify host targets involved in viral pathogenesis, perform genetic screens to identify host proteins important for infection, and then find drugs that inhibit these proteins. Rob discussed his lab's drug repurposing efforts and lessons learned about distinguishing useful hits from off-target outcomes based on their work on Ebola virus and SARS-CoV-2. Currently, there are no small molecule therapeutics for any of the

filoviruses. Ebola was one of the first viruses to be evaluated with repurposing libraries. Rob's group identified numerous active hits, of which many worked well to block infection; however, the mechanism of inhibition was ambiguous because these hits represented many classes of compounds. The group also identified hits for SARS-CoV-2 through screening studies and was surprised by the overlap between drugs that inhibit Ebola virus and SARS-CoV-2. While the viruses are not similar, many of the identified small molecules had comparable features (high pH, hydrophilic end to potentially interact with membranes, etc.) that were recently identified by others as inducers of phospholipidosis (suggesting a useful counterscreen assay), knowledge that could better direct selection of broadly acting compounds. While there are challenges to drug repurposing screens, Rob's group's work supports the success of this approach, citing, for example, their identification of high potency Ca^{2+} channel blockers (e.g., tetrandrine) that can potently block Ebola virus infection and confer high-level protection in mice.

Rob then discussed network analysis performed to relate drug action and virus-host protein interactions. This allowed the group to relate mass spectrometry data for protein-protein interactions (viral disease-ome) to the disease process and, ultimately, to treatment approaches. The group used the Broad Institute DRH library to identify drugs that can interact with "covidome" proteins. They learned how small molecules were structurally related, found scaffolds of related drugs, and identified hits that stood out based on their effect on various cellular functions (e.g., sensing Ca^{2+} levels). Some interesting standouts identified included inhibitors of BET, heat shock proteins, and PERK, and a BCL-2-apoptosis inhibitor (obatoclax).

Overall, Rob provided several examples of the utility of drug repurposing efforts and emphasized that success of this strategy is strengthened by an integrated approach that brings together knowledge of host targets of drugs, virus-host dependencies, and clinical usefulness/dosage regimens.

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

Laura Cooper described a design of novel 2-phenylthiophene derivatives as nanomolar inhibitors of SARS-CoV-2 papain-like protease (PLpro), which, upon binding, were able to induce new interactions with PLpro, i.e., the binding of the inhibitor to the BL2 groove mimicked the binding interaction between ubiquitin and PLpro.

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

Donghoon Chung presented the design of new, robust high-throughput screening (HTS) assay based on using FRET to measure the dsDNA-unwinding activity of SARS-CoV-2 helicase (Nsp13). Conducting an HTS assay of the Scripps Drug Discovery Library and confirmatory assays, including a dose-response study and an alternative fluorophore, identified a collection of chemically diverse and traceable scaffolds as potent hits targeting

viral helicases. These hits are currently being evaluated in cell-based antiviral assays with SARS-CoV-2 and other related viruses.

9.5. The Viral Non-Structural Proteins as Antiviral Targets: From Screening to Hit Validation. Bruno Canard, Ph.D., AIX Marseille University, Marseille, France

Bruno Canard presented the development of efficient screening assays against coronavirus and flavivirus replication complexes based on the activity of ATPase, methyltransferase, and polymerase, as well as protein-protein interactions. These assays represent a powerful screening platform for hit identification and subsequent hit-to-lead process. Various biophysics techniques, like thermal shift assays, were used to map hits and validate targets.

9.6. Antiviral Activity of Viperin-Inspired 3′-Deoxy-3′,4′-Didehydro-Nucleoside Phosphoramidate Prodrugs. Samantha Kennelly, Ph.D. Seeking, Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States

Samantha Kennelly reported that activation of the enzyme viperin results in broad-spectrum activity against DNA and RNA viruses via formation of 3′-deoxy-3′,4′-didehydrocytidine-5′-triphosphate (ddhCTP). The nucleoside ddhC in the form of ProTides exhibited significant antiviral activity in West Nile and Zika virus infection models, with favorable toxicity profiles, thus showing great potential as a potent and broad-spectrum antiviral.

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

Dominik Kiemel explained that the recently described anti-dengue virus compound JNJ-A07 targets an interaction between the viral protease/helicase complex NS2B/NS3 and the cleavage precursor NS4A-2K-NS4B and arrests the formation of vesicle packets, unravelling a novel antiviral mechanism of this compound.

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

Mikael Feracci explained that compound AT-752, active against dengue virus, is a prodrug that is metabolized into the active molecule AT-9010, 2′-methyl-2′-fluoroguanosine-5′-triphosphate. AT-9010 targets two activities of enzyme NS5: the RNA 2′-O-methyltransferase and the RNA-dependent RNA polymerase at its RNA elongation step.

9.9. Five Cellular Enzymes in the Activation Pathway of Bemnifosbuvir, a Drug-Candidate Against SARS-CoV-2 Infections. Aurélie Chazot, M.S., AFMB UMR 7257, Marseille, France

Aurélie Chazot started by describing bemnifosbuvir (AT-527), a nucleotide analogue prodrug that has recently entered Phase III clinical trials for the treatment of COVID-19. AT-527 is converted intracellularly into its triphosphate form, AT-9010, which targets the SARS-CoV-2 Nsp12 gene product, inhibiting both its viral RNA-dependent RNA

polymerase and nucleotidyltransferase activity. She detailed the proposed metabolism of AT-527 into AT-9010 and discussed her work ascertaining the active enzymes in this process and determining the order of the metabolic steps. After identifying five enzymes in the metabolic process (CatA, HINT1, ADALP1, GUK1, and NDPKB), her group expressed and purified them, and qualitatively and quantitatively confirmed their ability to catalyze their respective metabolites. The development of a standardized enzymatic assays allowed elucidation of the order of steps and identified GUK1 as the most critical enzyme in this pathway due to its specific substrate recognition system. The group also obtained two crystallographic structures (GUK1 and NDPKB), including that of a reaction intermediate, to describe the most likely mode of action of these key enzymes. The specificity of some key enzymes together with crystallographic structures of enzyme/substrate co-complexes point to new possibilities for designing improved nucleotide analogs and should help the understanding and use of the most relevant cellular, tissue, and animal models.

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

Abbie Weight began by describing the mechanism of action of BDAA, an orally available YFV antiviral. The primary mechanism of BDAA-mediated inhibition is via its interactions with NS4B, which directly inhibits viral replication; however, BDAA also appears to cause release of YFV viral replication intermediates (dsRNA), which increases activation of dsRNA-sensing pathways like RIG-I. In Syrian hamsters, the primary animal model for YFV, administration of 200 mg/kg/day BDAA, initiated 4 h pre-infection or 2 days post infection (dpi) and continuing for 7 days significantly improved survival, serum viremia, weight change, and serum alanine aminotransferase (ALT) concentrations. Treatment initiated 4 h pre-infection and 2 dpi both resulted in 100% survival compared to 100% mortality in placebo-treated hamsters. BDAA treatment initiated at 2 dpi resulted in a 2.5 $\log_{10}$-fold decrease in viremia compared to placebo controls. Histopathology showed decreased necrosis in liver tissue when treatment was initiated 2 dpi in comparison to placebo-treated animals and no visible liver necrosis in prophylactic treatment. Due to the lack of hamster-specific reagents, the group also performed comparable experiments in the $IFNAR^{-/-}$ mouse model, in which mock-treated mice demonstrated moderate clinical signs in the absence of mortality. In this model, no elevation of dsRNA-sensing pathway-related cytokines was seen 4 or 6 dpi when BDAA treatment was initiated 2 dpi, but significant elevation of CCL5, TNF-α, and IL-5 levels was seen 12 h after a single 4 dpi dose of BDAA, suggesting that a transient elevation in specific cytokine concentrations occurs briefly after treatment is initiated during established YFV infection. These results further demonstrate BDAA in vivo efficacy against YFV and support the potential of NS4B inhibitors as treatments for YFV.

9.11. New Class of Small Molecules That Inhibits Yellow Fever Virus by Targeting the NS4B Protein. Alina Soto, Ph.D. Seeking, KU Leuven, Department of Microbiology,

Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium

Alina Soto began by giving background on YFV, its associated disease, and the urgent need for the development of safe and effective antivirals. Alina then described her work identifying a new class of synthetic YFV inhibitors targeting the NS4B viral protein. From a series of heterocyclic compounds with hexahydro-2H-4,6-(epoxymethano) chromene moieties, compound I7–20-1 had potent antiviral effect in two cells lines (Huh7 and Vero cells) against YFV with minimal cytotoxicity. No antiviral activity was detected against Zika virus (flavivirus), Chikungunya virus (alphavirus), or enterovirus-71 and human rhinovirus 14 (picornaviruses), suggesting YFV-specific antiviral activity. Time-of-addition experiments showed that the antiviral effect of I7–20-1 was highest when given up to 4 h post infection, suggesting that I7–20-1 acts early in the replication cycle. To elucidate the molecular target of I7–20-1, a resistant YFV variant was selected with a >25-fold resistance to this compound. A single mutation (I84T) was identified in the NS4B gene. The mutated isoleucine is located within the transmembrane domain 2. Other YFV NS4B inhibitors (BDAA and CCG-4088) were previously described, but resistance was reported in different regions of NS4B, suggesting different mechanisms of action. Alina finished her talk describing ongoing experiments with cross-resistance assays and work to reverse-engineer the YFV NS4B-I84T mutant.

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

Dahai Luo began by describing his work with Chikungunya virus replication machinery and elucidation of how positive-strand (+) RNA viruses assemble membrane-associated replication complexes (RC) to synthesize, process, and transport viral RNA in virus-infected cells. Using in vitro reconstitution and in situ electron cryotomography, Dahai's group determined both the high-resolution structure of the core RNA replicase of Chikungunya virus and the native RC architecture in cellular context at sub-nanometer resolution, respectively. He detailed several experiments that ultimately showed how the core RNA replicase, the viral polymerase nsP4 in complex with nsP2 helicase-protease, sits in the central pore of the membrane-anchored nsP1 RNA-capping ring. The addition of a large cytoplasmic ring next to the C-terminus of nsP1 forms the holo-RNA-RC as observed at the neck of spherules formed in virus-infected cells. Dahai finished by discussing how better understanding of the molecular basis of viral RNA replication within the RC will serve as a useful tool for developing antivirals against alphaviruses and other (+) RNA viruses, since the principles underlying the molecular architecture of RCs are likely conserved.

9.13. The MEK1/2 Inhibitor Zapnometinib Is Safe and Well Tolerated in Humans and Has Both Anti-SARS-CoV-2 as Well as Immunomodulatory Activity. Oliver Planz, Ph.D., Eberhard Karls University, Tübingen, Germany

The cellular Raf/MEK/ERK signaling pathway is hijacked by many viruses, like influenza virus and RSV, to ensure their propagation. The pathway is also involved in regulating cytokine and chemokine responses. **Oliver Planz** discussed how targeting this pathway to treat viral infections can have a dual effect, with both antiviral activity and

immunomodulation of both innate and adaptive responses. He illustrated this mechanism by sharing his group's studies demonstrating the efficacy of zapnometinib, an inhibitor of the host cell kinase MEK, against SARS-CoV-2. In vivo studies confirmed high level efficacy of zapnometinib treatment against SARS-CoV-1, MERS, and all variants of SARS-CoV-2 tested in vitro. They then used the SARS-CoV-2 hamster model to demonstrate viral load reduction in nasal turbinates and protection from severe lung damage with zapnometinib treatment. The mechanism of action had been previously described as causing retention of viral RnP complexes in the nucleus of influenza virus-infected cells. However, for SARS, which does not have a nuclear phase, the antiviral activity likely results from interference with entry. Oliver continued by sharing results from the Phase I study that found zapnometinib to be safe and well tolerated and to have promising pharmacokinetics. Finally, Oliver shared Phase 2 study data showing that viral loads were reduced in nasal and sputum samples from SARS-CoV-2-infected, hospitalized patients (RESPIRE trial; NCT04776044). Altogether, his group concluded that these data support continued studies of zapnometinib for treating SARS-CoV-2, for potential use as a broad-spectrum antiviral drug, and for pandemic preparedness.

9.14. AI-Driven Approach to the Discovery of Novel M^{pro} Inhibitors with High Pan-coronavirus Activity. Marco Derudas, Ph.D., Exscientia, Oxford, United Kingdom

Marco Derudas spoke about Exscientia's research to develop once-daily oral antiviral therapeutics with broad-spectrum activity against SARS-CoV-2 variants, other human coronaviruses (i.e., MERS-CoV, SARS-CoV), and putative pre-emergent bat coronaviruses. The SARS-CoV-2 main protease (M^{pro}), highly conserved among human coronaviruses, was identified as a target for an artificial intelligence- (AI) driven drug discovery effort by an internal tractability analysis. Compound design was driven by generative design algorithms. Subsequently the compounds were scored and filtered, deploying machine learning models to select optimal molecules for synthesis and testing based on potency, selectivity, and drug-like properties. Lead optimization was focused on improving selectivity over human proteases, increasing metabolic stability and absorption, and mitigating risk of drug-drug interactions. Ultimately, highly potent, broad-spectrum M^{pro} inhibitors (like EXS0191190 and EXS0186845) were identified and tested against both human and bat coronaviruses, demonstrating their potential for broad application to treat current and future outbreaks.

10. Session 7: acute gastrointestinal viruses

Chaired by Brian Gowen and Joana Rocha-Pereira.

10.1. What Viruses Do to Infect You! (And Why This Is Important for Antiviral Strategies). Nihal Altan-Bonnet, Ph.D., National Institutes of Health, USA, Bethesda, Maryland, United States

Nihal Altan-Bonnet started by explaining that her talk was composed by two parts: the first focusing on cell-to-cell transmission, specially addressing *en bloc* virus transmission (when multiple viruses are transported in an extracellular vesicle (EV) to infect another cell), and the second discussing new routes of organism-to-organism transmission. Nihal challenged the conventional view on viral transmission, in which new viruses emerge and spread as

freely moving independent particles and one virus particle is sufficient to infect and replicate in a host cell. Her research showed that multiple enteroviruses can exit host cells inside extracellular vesicles; this finding has been expanded (also by other groups) to include a wide range of human viruses, both RNA and DNA viruses, enveloped and non-enveloped. Importantly, she demonstrated that vesicle-cloaked viral inoculums are more infectious than free-floating virions because they reach a certain input threshold to replicate in vitro. This input threshold is ~15 virus particles for poliovirus and ~80 virus particles for SARS-CoV-2 D614G, which is just about the number of particles often found in a vesicle (poliovirus) or aggregate (SARS-CoV-2).

Nihal then went on to show that vesicles can transmit infections among organisms, using suckling pup animal models of diarrhea-causing viruses like rotavirus, norovirus, and astrovirus, which collectively infect 1.5 billion people yearly, are a major cause of mortality and morbidity associated with enteric disease. Notably, fluorescently labeled vesicles could still be found in the intestines of pups, attesting to their stability passing through the gastrointestinal tract. Nihal pointed out that disrupting *en bloc* transmission of viruses by means of small molecule inhibitors would be a valuable antiviral strategy, either by designing molecules that target the vesicles (or viral aggregates) themselves or the cellular pathways involved in their production.

These gastrointestinal viruses were assumed to be transmitted solely by the fecal-oral route, but Nihal's group recently identified a new route of transmission, via saliva. When these suckling pups were infected with these viruses and then placed back with their mothers for suckling, the researchers observed a rapid rise in secretory IgA in the intestine of the pups and also in their mothers' milk. Nihal's team went on to discover that the mammary glands of the mothers become infected due to backflow of pup saliva during suckling. High viral loads were found both in saliva and salivary glands of infected pups, and this saliva could be used to infect new animals. Infection cleared more quickly in pups whose salivary glands were removed, suggesting that the intestinal infection is fed by saliva and that the salivary glands likely are a viral reservoir. This finding has implications for virus control, as blocking salivary transmission is thus necessary to contain the spread of these viruses within a household, for example. In addition, this finding raises questions regarding the existing commonalities between the multiple infected tissues: salivary glands, mammary glands, and intestines, such as common viral receptors, viral input thresholds for replication, or similar tissue microenvironment.

Nihal ended her talk by emphasizing that for 300 million years mammals have been suckling on their mothers, so mammary glands and salivary glands have been in close proximity and probably undergoing co-evolution. This connection has long been overlooked but should be studied and given more attention, as her work suggests this is a relevant bidirectional communication route.

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

Marcella Bassetto started with an overview of her research group (collaborating with Dr. Salvatore Ferla) and work on identifying norovirus polymerase inhibitors. Her group used computational approaches to identify new biochemical hit compounds of the norovirus polymerase, and then applied *in silico* and traditional medicinal chemistry approaches to convert them into antiviral compounds. Noroviruses are a major cause of foodborne illness and cause extensive outbreaks of gastroenteritis, being responsible for significant mortality, mainly in children in developing countries. Norovirus infections cause also significant economic losses; for example, in the UK, they are a major reason for closure of hospital wards, with an annual cost of ~£81 million to the health service. Currently there are no vaccines or antivirals to treat or prevent norovirus infection.

The norovirus polymerase (RdRp) has been Marcella's group's selected target due to its essential role and conservation across the diverse norovirus genogroups and genotypes. Since abundant structural information exists for this viral enzyme, a combination of structure and ligand-based virtual screening was performed with the aim of filtering commercial libraries of drug-like molecules and identifying those with a best predicted binding to the enzyme. Next, biochemical assays were performed and two chemical scaffolds were selected, around which ~50 new molecules were synthetized. Given the broad effect of the molecules against multiple calicivirus polymerases, Marcella's group hypothesized that the molecules could bind a conserved site within this polymerase. Antagonism with PPNDS, a known RdRp site B binder, further corroborates this hypothesis. Molecular docking studies indicated that this compound class interacts with site B but also protrudes to site A. However, no activity in virus-infected cell-based assays was observed, likely due to the low solubility of the molecules. Chemical modifications to improve solubility together with a strategy of disrupting the planarity of the molecules and thus replacing the central linker using scaffold hopping resulted in inactive compounds.

Marcella then decided to take a step back and used a flexible alignment technique to perform a structural comparison with known non-nucleoside inhibitors of viral polymerases, which allowed her to design novel inhibitors that were active against human and murine norovirus (MNV) in cellular assays. Additional analogs were synthetized and contributed to delineation of the structure-activity relationships for this class of molecules, despite having issues of instability in aqueous solution and synthetic issues due to formation of side products in the last synthetic step. Further efforts to solve these problems led to the identification of the first molecule of the series with sub-micromolar activity. The generation of 3D-QSAR models for the antiviral activity of the molecules will guide further efforts of optimizing the potency and overall characteristics of this class. Importantly, the activity of the class has been confirmed in human norovirus GII.4-infected human intestinal enteroids (in the lab of Dr. Rocha-Pereira, at KU Leuven), a complex and highly physiologically relevant model with intermediate complexity between in vitro and in vivo.

Lastly, Marcella introduced a new project aiming to target the NS1–2 non-structural protein of noroviruses, particularly due to the presence of a catalytic triad analogous to that of papain-like thiol peptidases in its C-terminal portion. Preliminary data showed antiviral activity for one hit molecule, identified by virtual screening of commercial libraries. Additional efforts are ongoing to improve activity and define the structure-activity relationship.

10.3. Anti-Hepatovirus Activity of TENT4A/B Inhibitors. You Li, Ph.D., University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

You Li started by addressing the importance of hepatitis A virus-(HAV) induced viral hepatitis and highlighted the fact this virus circulates in blood as a “quasi-enveloped” virus, while it is shed in stool in a “naked” non-enveloped form. While the introduction of a vaccine has helped to control the number of infections, resurgent community outbreaks have been reported and justify the need for specific antiviral therapies.

While hepatitis A virus (HAV) is not phylogenetically related to hepatitis B virus (HBV), the team of You and Dr. Stanley Lemon found that RG7834, an antiviral active against HBV, is also highly potent against HAV in vitro and in an IFNAR knockout mouse model. Serum ALT values, inflammation, and vRNA levels in stool were strongly reduced by treatment with 10 mg/kg twice per day, although some viral rebound was noted at late time points. You went to investigate why an HBV antiviral has anti-HAV activity.

A CRISPR screen for HAV host factors identified ZCCHC14, a zinc finger protein, as important for viral replication; a few years later, the same protein was found to also be important for HBV replication. ZCCHC14 was shown to bind a stem-loop in HBV RNA to which it recruits non-canonical poly-A polymerases TENT4A and 4B. The complex formed by ZCCHC14-TENT4 promotes HBV replication by elongating the 3′-poly-A tails of HBV viral RNAs. You studied the role of this complex in HAV replication and discovered that ZCCHC14 binds to HAV’s 5′-UTR, specifically to stem-loop Vb, but is not involved in IRES-mediated translation or polyprotein processing. The inhibitor RG7834 suppresses HAV RNA synthesis, and thus ZCCHC14-TENT4 may support the circularization of the viral genome, which is important for RNA synthesis. Indeed, TENT4 interacts with HAV’s 3′-UTR and RG7834 disrupts the ZCCHC14-TENT4 interaction. The detailed underlying mechanism is still under investigation in You’s lab.

Clinical development of RG7834 was started by Roche but halted after a Phase I trial due to toxicity after prolonged exposure in animals. Likewise, neurotoxicity of a structurally related molecule was reported after 14-week rat and monkey studies. Short treatment periods or safer chemical scaffolds are preferred for further development. Collaborators at Harlingene Life Sciences are currently improving the pharmacokinetic/pharmacodynamic properties of this class of compounds.

11. Session 8: chronic, persistent, or latent viruses III

Chaired by Graciela Andrei and Jennifer Moffat.

11.1 Differential Dynamics and Evolution of Cytomegalovirus Infection in Transplant Recipients Grafted with Organs Derived from the Same Donor. Fien Horsten, Ph.D. Seeking, KU Leuven, Leuven, Belgium

Fien Horsten, a Ph.D. student from KU Leuven, introduced HCMV and pointed out that available antiviral therapy has limitations due to emergence of drug-resistant variants. Within RegaVir, a translational research platform, the evolution of HCMV infection was studied in three patients who received a transplant from the same donor on the same day. These three patients had the same UL97 protein kinase and DNA polymerase natural genetic polymorphisms, as well as a novel A505G substitution. A differential evolution of the CMV donor strain was observed with development of ganciclovir resistance in two of the patients, but due to mutations in different genes. Whole-genome HCMV sequencing was performed and is now being analyzed in depth.

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

Julio Cesar Villalvazo Guerrero explained the clinical importance of HSV-1 and HSV-2 and the limitations of currently available antiviral therapies. After screening 19,000 compounds using an HSV reporter virus, two molecules were selected for further studies: PANH-135 and PANH-070. These showed good activity against acyclovir-resistant variants and a murine skin ex vivo model. Using a reporter strain in which a fluorescent protein is fused to the HSV-1 capsid, the effect of both compounds was equipotent but different, suggesting different modes of action. While PANH-135 inhibited the formation of C-capsids, PANH-070 reduced the budding of the capsids into the inner nuclear membrane.

11.3. Herpesvirus-Mediated Protein Citrullination as a New Target for Antiviral Therapy. Selina Pasquero, Ph.D., Department of Public Health and Pediatric Sciences, University of Turin Medical School, Turin, Italy

HSV-1 infections are treatable with acyclovir and its derivatives, but drug resistance may arise. More potent drugs are needed, especially for HSV-1-caused encephalitis. Targeting host functions is a promising direction for new drug development. In this presentation, **Selina Pasquero** described the interaction of HSV-1 with peptidylarginine deiminases (PADs), which convert arginine to citrulline in proteins. The rationale for investigating this was her group's recent publication in *Nature Communications* showing that another herpesvirus, HCMV, induces PADs. Selina's group labeled proteins in HSV-1-infected cells with a rhodamine phenylglyoxal (Rh-PG) probe that specifically binds to citrulline in proteins and is detectable on blots and by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Many HSV-1 proteins were citrullinated in infected cells, including gD. Many host proteins were also differentially citrullinated, including IFIT1 and IFIT2. PAD3 protein was strongly induced in infected cells. PAD inhibitors Cl-amidine and BB-Cl-amidine prevented HSV-1 replication with an IC_{50} of 0.7 μM for BB-Cl in HFFs. PAD3 inhibitor HF4 had an IC_{50} of 0.5 μM. When PAD3 was knocked down with siRNA, HSV-1 titers were reduced by 3 logs.

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

The Institute of Medicine has prioritized a vaccine against HCMV because congenital CMV infection (cCMV) is a major health concern, causing cognitive impairment and hearing loss, among other problems. **Alistair McGregor** is addressing the requirements for protective vaccination by studying immunity to experimental CMV vaccines in a guinea pig model of congenital infection. Since cytomegaloviruses are species restricted, his research employs guinea pig CMV (GPCMV). A laboratory strain of GPCMV, 22122, has long been used for this type of work. In fact, this is the 100th year of GPCMV research! The purpose of the presented study was to evaluate GPCMV vaccines for protecting guinea pig pups against congenital infection by strain 22122 and a newer strain, TAMYC, that is highly cell associated. Their previous work on cCMV vaccines showed that a disabled infectious single cycle (DISC) vaccine strategy completely protected against congenital GPCMV 22122 strain but not against TAMYC. They adopted adenovirus-vectored vaccines as a new strategy. The gB glycoprotein expressed as a trimeric complex (AdgB vaccine) induced an antibody response that protected against 22122 but was less effective against cell-associated TAMYC. Then T cell targets were selected; GP83 is the pp65 homolog and GP123 is the IE1 homolog. Both induced T cell responses in ELISPOT assays. AdGP83 and AdIE1 vaccines protected against 22122 dissemination but protection was limited against TAMYC. A double antigen strategy was then tested against congenital infection, which included AdgB + AdIE1. The combination was compared to AdgB or AdIE1 single vaccines and unvaccinated animals. Guinea pig dams were challenged while pregnant with 22122 and TAMYC together, then the pups were analyzed for congenital infection. The double vaccine strategy, combining surface antigens with a T cell antigen, resulted in full protection of all 36 pups and no virus was detected. AdgB alone was 88% effective but virus was detectable in the placenta and some pups. AdIE1 alone was also protective but not as potently as the combined vaccine.

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

HSV-1 and HSV-2 cause infections worldwide and current treatment options do not prevent reactivation or eliminate reservoirs. Latency in neurons is regulated, but the mechanism of this regulation is not totally known. Viral chromatin is highly dynamic during lytic infection, whereas during latency, the HSV-1 genome is assembled into stable chromatin and only the latency associated transcript is made. Chromatin remodeling complexes are essential for cellular genome integrity and expression and during DNA replication repair and transcription, but their roles during viral infection are unclear. Four BAF complexes were investigated by **Sarah Saddoris**'s group using small molecule bromodomain inhibitors. Five inhibitors were selected: PFI-3 inhibits the bromo domain in the common SMARCA2/4 subunit in cBAF, PBAF, and GBAF; I-BRD9 inhibits the unique BRD9 subunit; and LP99, TP-472, and BI-9564 inhibit the BRD9 and BRD7 subunits. The effects of these inhibitors

on HSV-1 replication were assessed in HFF and HeLa cells, and all but TP-472 increased virus yield. TP472 increased HSV-1 yield when used at low concentration and reduced viral yield at high concentration. None of the inhibitors reduced HSV-1 DNA levels at late times with statistical significance. These results led to a model in which PBAF promotes HSV-1 replication and GBAF inhibits it. HSV nuclear domains (HND) were observed by immunofluorescence microscopy, and SMARCA4, SMARCA2, and SMARCC1 all colocalized with the HND. BRD9 and ARID1A also colocalized with HND, but BRD7 did not. A method was developed to quantify the colocalization results by measuring overlapping pixels. This analysis showed that BRD7 colocalized to HND the least. Histone acetylation marks, which are recognized by bromodomains, were depleted from HND, with H3K36 the most depleted. The working model is that BAF complexes modulate HSV-1 infection, some BAF subunits are enriched in HND, and recruitment of BAF subunits into HND does not depend on the bromodomains targeted by small molecule inhibitors. Future work will determine whether BAF complexes assemble during HSV-1 lytic infection, if they affect viral gene expression, or if they bind viral genomes.

11.6. POM-L-BHDU Is a Highly Potent Prodrug of L-Dioxolane Bromovinyl Uridine That Prevents Varicella Zoster Virus Spread Topically in Skin Organ Culture and Orally in NuSkin Mice. Megan Lloyd, Ph.D., SUNY Upstate Medical University, Syracuse, New York, United States

VZV causes chicken pox and shingles, and there is a critical need for more drugs to treat these diseases. VZV is restricted to humans, so antiviral compounds are tested in human skin models. Adult skin is obtained from reduction mammoplasties and then is thinned and cut into 1 cm^2 pieces. The pieces of skin are either placed on NetWells for culture or implanted subcutaneously in nude mice (NuSkin model). Skin explants can be used immediately; xenografts are vascularized over 4 weeks in mice. Skin is infected by scarification with a VZV strain that expresses firefly luciferase, and virus spread is measured by bioluminescence imaging. Megan Lloyd talked about using these models to evaluate POM-L-BHDU (POM), which is a phosphoramidate prodrug of L-BHDU. The purpose of this project was to determine whether POM-L-BHDU is safer and more effective than L-BHDU. In ARPE-19 cells, the EC_{50} of POM was 0.03 μM and the CC_{50} was greater than 100 μM, producing a selective index >2800; this potency is similar to that of L-BHDU. POM and L-BHDU were as effective as acyclovir in the skin organ culture model when applied topically. Histopathology of H&E-stained sections showed that that POM and L-BHDU were not cytotoxic to skin. In NuSkin mice, POM was effective subcutaneously and orally while L-BHDU was not; POM was effective at doses as low as 11.3 mg/kg and was well tolerated in mice even at 45 mg/kg. A pharmacokinetic analysis of oral administration showed that POM was rapidly metabolized to L-BHDU and its half-life in plasma was 5.6 h. When equimolar doses of L-BHDU and POM were given orally, POM accumulated at higher levels in plasma than did L-BHDU. In vitro, POM was stable in gastric and intestinal fluids.

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

HSV-2 is one of the most common sexually transmitted infections. Genital lesions can be treated with nucleoside analogs, but there is no sterilizing cure. More drugs are needed. Oxysterols are 27 carbon molecules derived from cholesterol oxidation, and they have broad-spectrum antiviral activities. The purpose of **Andrea Civra**'s study was to screen a library of oxysterol synthetic analogs (named PFMs) for activity against HSV-2 and investigate their mechanisms of action; 17 PFM compounds were selected for the first screen. PFM064 was identified as an HSV-2 inhibitor. In the second round of testing, compounds with similar structure were selected; two compounds, PFM067 and PFM069, had activity against HSV-2, which was confirmed in yield reduction assays. Their potency was in the low micromolar concentration, and the SI were above 100. PFM067 was the most promising compound, so its mechanism of action was investigated. Time of addition assay showed that PFM067 reduced the size of syncytia when it was added 18 h after infection. This effect was linked to antiviral activity and was dose dependent. Immunoblots of cytoplasmic proteins from infected cells showed low amounts of VP5, a capsid protein, while glycoprotein levels were normal. Transmission electron microscopy confirmed that capsids were retained in the nuclei of treated cells. Furthermore, few infectious virions were produced. A working hypothesis was developed that PFM067 acts on late replication steps and prevents virion maturation. Immunofluorescence microscopy showed gH/gL in the center of syncytia that colocalized with a Golgi marker. In treated cells, all glycoproteins were sequestered in the Golgi and were not in the plasma membrane. Capsids were restricted to nuclei in treated cells. These promising results will be pursued in future work addressing the antiviral target of PFMs and whether they are active against other herpesviruses. The group is initiating target-based synthesis of novel oxysterols with anti-herpetic activity.

12. Shotgun presentations

For the final session of ICAR 2023, six presentations were selected from the poster abstracts as 10-min "Shotgun" presentations: Patrick Tate, B.S., "Peptoid amphiphiles as membrane active antivirals"; Calvin Gordon, Ph.D. seeking, "Efficient incorporation of 2′-fluoro,2′-bromouridine triphosphate inhibits yellow fever virus polymerase selectively"; Ashleigh Shannon, Ph.D., "A non-excisable nucleotide analog active against SARS-CoV-2"; Katherine Davies, Ph.D., "Generation and characterization of recombinant MA-EBOV expressing reporter proteins in vitro and in vivo for use in therapeutic and vaccine efficacy studies"; Noemie Berry, Ph.D., "Modeling SARS-COV-2-infected central nervous system using human primary neuronal/glial cells to identify antiviral drugs"; and Ben Flude, Ph.D. seeking, "Targeting the interaction between host MASP-2 and the viral N protein as a broad-spectrum therapeutic approach for coronavirus infections."

13. Women in Science forum

The annual Women in Science forum was held prior to the opening session of ICAR 2023. This year's event featured a discussion led by panelists Joanne Lemieux (University of

Alberta), Jessica Spengler (US CDC), Jennifer Moffat (SUNY Upstate), and Joana Rocha Pereira (KU Leuven), and was hosted by Kara Carter (Evotec). Joanne Lemieux was also the recipient of the 2023 ISAR Women and Excellence in Science Award. The discussion focused on the findings of the Tallest Poppy Study (http://www.womenofinfluence.ca/2023/03/01/tps-press-release/). Namely, this study found that senior women faced the following obstacles in their careers:

- Their achievements were downplayed.
- They were left out of meetings and discussions or were ignored.
- They were undermined because of their achievements.
- Their achievements were dismissed.
- Others took credit for their work.

The panelists shared personal experiences that exemplified these obstacles and discussed their impact and ways they had found to either avoid or clear these obstacles. Characteristically of this annual event, attendees included both women and men, those early in their career as well as those farther along. Honest questions and candid sharing occurred throughout the event. As in the past, attendees expressed great appreciation to the panelists, audience participants, and ISAR for continuing to support this important event.

14. PechaKucha competition

This year's PechaKucha contest was, as always, one of the highlights of the ICAR program. Seven brave contestants took the stage to impress us with their ability to convey key aspects of their research projects, as well as personal information about themselves, all while using humor and adhering to the strict PechaKucha rules. For those not familiar with the format, it was invented by two architects in Japan in 2003 and requires the speaker to present using 20 slides at 20 s each and the speaker has no control over the movement of the slides, so they must practice and time themselves perfectly. We have adapted it slightly due to time constraints and the graduate students or postdocs only have 15 slides at 20 s each to tell their stories. Just as important as the timing is the use of humor or some sort of theme - another critical aspect of the format is entertaining the audience while still getting across the information.

As many previous ICAR attendees remember, we have had numerous themes over the past few years, including James Bond, Lord of the Rings, Star Wars, Star Trek, Game of Thrones, wizards, and a jungle explorer, and so it was no surprise that this year we saw several very clever costumes and themes for the talks, including Sarah Saddoris who dressed as a sandworm from Dune. Overall, it was a fun and entertaining segment of the ICAR program, and we congratulate the winners!! In first place ($250) was Michelle Law from Nanyang Technological University, who regaled us by mentioning a number of famous Michelles, including the latest Oscar winner, and her hope to follow in their footsteps and ultimately become famous too by winning a Nobel prize. She was followed by our $150 s place winner Gregory Mathez from the Institute of Microbiology in Switzerland, who tied in various kinds of chocolates with his own work, i.e., "Greg's sugars", and even had a

container of chocolates that he offered to hand out at his poster! In third place, winning $75, was Julio Cesar Villalvazo Guerrero from the Hanover Medical School in Germany, who entertained us with various Mexican references including the increasing number of sombreros on each of his slides. Finally, our two runners up, Sarah Saddoris (Cornell University) and Scott Gibson (Institute for Antiviral Research, Utah State University), were given the special honor of chairing the shotgun talks during the closing session of ICAR 2023. Congratulations again to all our winners and we look forward to seeing next year's contestants in Gold Coast, Australia, in May 2024!

15. Career development interactive roundtable

The ICAR career development session this year was an interactive Career Roundtable. The Career Roundtable aims to give attendees the opportunity to meet established researchers who provide their unique perspectives on career development, professional pitfalls, and scientific opportunities for trainee scientists. We were grateful to have a group of excellent, experienced researchers, reflecting a myriad of career paths and experiences (academia, industry, government, NGO, etc.), who were so kind to attend the roundtable: Steve Polyak, Cybele Garcia, Subhash Vasudevan, Xavier Manière, Kara Carter, John Billelo, Olivia Goethals, Tina Thorslund, Rob Jordan, Ashish Pathak, Maaike Everts, Jinhong Chang, ad Nicolette Van Dijk, and Luis Schang.

The ICAR Career Roundtable took place on Tuesday during lunch in the Roseraie rooms. The session started with a light lunch, followed by a short explanation of the concept and an introduction of the senior scientists. Eleven round tables were set up and organized by different career paths, with each table seating one or two senior researchers. Four rounds of 15 min were organized, during which attendees could choose a table of their interest. At each table, attendees were able to interact with the senior scientists in a comfortable, small group setting. The Career Roundtable was followed by a short, informal networking moment.

The Career session was well attended this year, with 50 participants (and many attendees on the waiting list). The career development committee would like to thank the senior scientists for being willing to share their stories and advice, and all the participants for their enthusiastic attendance!

16. Poster awards

A total of 204 posters were accepted for ICAR 2023; 183 of them were presented at two in-person poster sessions and an additional 21 were included on the virtual platform. Both sessions were well attended, with much interaction and interesting discussions between poster presenters and other attendees. A record number of 83 posters were competing this year to win a poster prize in one of the following categories: (1) graduate student, (2) post-doctoral researcher, and (3) young investigator (junior faculty member or the equivalent). The posters in the competition were visited by at least 2 poster judges. More than 20 senior scientists volunteered to judge posters. The quality of the entries was incredibly high.

In the graduate student category, the following prizes were awarded: Jazmin Galvan Achi for, “Development of small molecule entry inhibitors of influenza A viruses,” (first prize, $500); Patrick Tate for, “Peptoid amphiphiles as membrane active antivirals,” (second prize, $400); Viriginia Aida-Ficken for, “Identification and evaluation of novel macrocyclic compounds against hemorrhagic fever arenaviruses,” (runner up, $250); Calvin Gordon for, “Efficient incorporation of 2′-fluoro,2′-bromouridine triphosphate inhibits yellow fever virus polymerase selectively,” (runner up, $250); Arryn Owens (who is an undergraduate student!) for, “HSV-1 chromatin is enriched in highly dynamic histone variant H2A.B in replicating and transcribed viral DNA,” (runner up, $250); and Janna Scherf for, “Druggability assessment of the bunyaviral cap-binding domain,” (runner up, $250). In the post-doctoral researcher category, two first-prize categories were awarded (each $750): Anuj Kumar for, “Cytosine base editing inactivates the hepatitis B virus episomal genomic reservoir and integrated DNA,” and Ashleigh Shannon for, “A non-excisable nucleotide analog active against SARS-CoV-2.” Finally, in the young investigator category, two first-prize categories were also awarded (each $1000): Malakia Argade for, “Small molecule entry inhibitors of Ebola and Marburg filoviruses,” and Stephen Welch for, “Single dose mucosal delivery of a Nipah VRP-based vaccine confers rapid protection against lethal disease.”

17. Chu Family Foundation awards

Three Chu Family Foundation awards were given in 2023 (each $3,000): to Rebekah Dickmander (graduate student, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA), Selina Pasquero (post-doctoral fellow, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy), and Joy Thames (Ph.D. student, University of Maryland at Baltimore County, Baltimore, MD, USA).

17.1. Concluding remarks and invitation to the 37th ICAR – Gold Coast, Queensland, Australia, May 2024

The 36th ICAR was a resounding success with record-breaking in-person, as well as total, attendance. All speaker sessions and special events were well attended and aided in continuing to strengthen the antiviral research community. On behalf of the 37th ICAR organizing committee, we would like to invite you to join us in Queensland, Australia. The 2024 meeting will be held on the sunny Gold Coast, home to the Gold Coast Health and Knowledge Precinct (GCHKP), Asia-Pacific’s newest biomedical innovation hub. Australia has a rich history of antiviral research and numerous active research programs focused on vaccine and antiviral efforts. This research community includes university-based programs across the country and additional public and private research initiatives, such as the newly established Translational Science Hub, a unique collaboration between Sanofi, the University of Queensland, Griffith University, and the Queensland Government. The Australian antiviral research community is honored to host this uniquely transdisciplinary conference, and welcomes chemists, virologists, biologists, clinicians, or anyone passionate about antiviral research. Mark your calendars and save the date: May 20–24, 2024, on the Gold Coast, where sun, sand, surf, and world-class research will come together. We will see you there!

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Data availability

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

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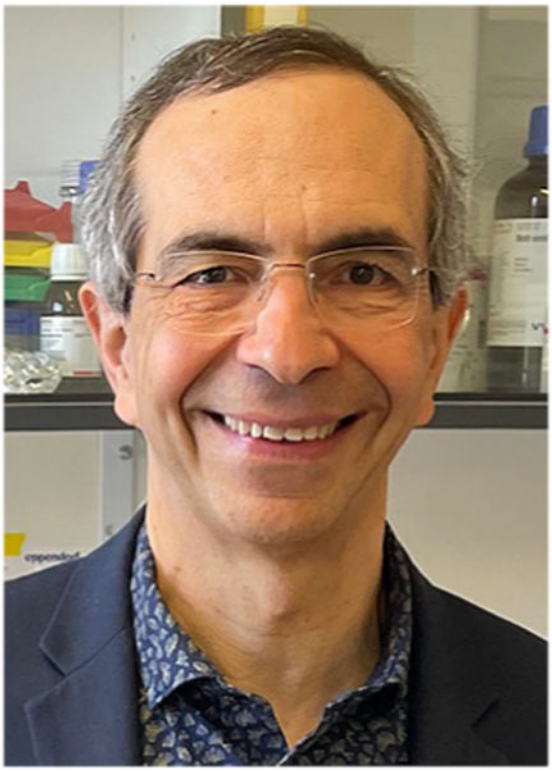

Fig 1.
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Fig 2.

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Fig 3.
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Fig 4.
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Fig 5.
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